

From The Department of Clinical Neurosciences  
Karolinska Institutet, Stockholm, Sweden

**GENOTYPE-PHENOTYPE CHARACTERIZATION OF FAMILIAL  
HYPERKINETIC MOVEMENT DISORDERS: EMPHASIS ON ATAXIA  
AND BRAIN CALCIFICATIONS**

Martin Paucar  
M.D.



**Karolinska  
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# Genotype-phenotype characterization of familial hyperkinetic movement disorders: emphasis on ataxia and brain calcifications

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

**Martin Paucar**

*Principal Supervisor:*

Professor Per Svenningsson  
Karolinska Institutet  
Department of Clinical Neuroscience

*Co-supervisor(s):*

Professor Magnus Nordenskjöld  
Karolinska Institutet  
Department of Molecular Medicine and Surgery

Professor Ann Nordgren  
Karolinska Institutet  
Department of Molecular Medicine and Surgery

Professor Maciej Machaczka  
University of Rzeszów  
Department of Medicine

*Opponent:*

Professor Susan Perlman  
University of California Los Angeles  
Department of Neurology at the David Geffen  
School of Medicine

*Examination Board:*

Professor Krister Kristensson  
Karolinska Institutet  
Department of Neuroscience

Professor Chantal Tallaksen  
University of Oslo  
Institute of Clinical Medicine

Associate Professor Joakim Tedroff  
Karolinska Institutet  
Department of Clinical Neuroscience



## ABSTRACT

Differential diagnosis of familial chorea encompasses Huntington's disease along with a group of conditions referred to as Huntington's disease-like (HDL). One such HDL is an inherited prion disorder (IPD) caused by pathological insertions of 8 additional OPRIS in the prion protein gene (*PRNP*). Only four 8-OPRI families have been reported, one of which was Swedish. Polymorphism in codon 129 of the *PRNP* gene, alternating between methionine (M) and valine (V), is the primary modulator of prion diseases. The Swedish family had the longest survival of any 8-OPRI family. Patients carrying 129M in the mutated allele demonstrated earlier age of onset (AO), longer survival and earlier age of death than those with 129V. *PRNP* polymorphism in codon 129 together with gender determined as much as 50% of the variability in AO. An inverse correlation between early AO and length of survival was observed (**Paper I**).

Ataxia with oculomotor apraxia type 4 (AOA4) is caused by mutations in the gene encoding polynucleotide kinase 3-prime phosphatase (*PNKP*) gene. A Swedish patient with AOA4 due to compound *PNKP* mutations, progressive symptoms and cerebellar atrophy was characterized (**Paper II**). Novel AOA4 features in this case were chorea during childhood, slower disease progression than previously described and low levels of the PNKP protein in her lymphocytes.

Spinocerebellar ataxia type 4 (SCA4), a rare disease first described in a Scandinavian family in the American Midwest in 1996 has been found to be linked to chromosome 16q22.1. A second SCA4 family was later identified in Germany. Two Swedish SCA4 families with novel symptoms such as dystonia and dysautonomia are described here. Symptom onset was at middle age and anticipation was suggested in one family. Variable infratentorial atrophy and spinal cord atrophy was evident in all the tested patients. Flumazenil-PET revealed reduced binding in several brain regions including the insula, thalamus, hypothalamus and cerebellum. The candidate region was sequenced but no pathogenic variants were found. Widespread neurodegeneration was exhibited by two cases (**Paper III**).

Primary familial brain calcifications (PFBC) are heterogeneous diseases. One Swedish-Finnish family (F13) with such calcifications and associated migraine, hyperkinesias and psychiatric symptoms associated is described along with five other PFBC families. The F13 family harbors the L9R mutation in the platelet-derived growth factor beta polypeptide (*PDGFB*) gene. Other *PDGFB* mutations were identified in the remaining families. A hypomorphic *PDGFB*<sup>ret/ret</sup> mouse model displays brain calcifications and an impaired blood-brain barrier (BBB). **Paper IV** established mutations in *PDGFB* as the second most common cause of PFBC, after mutations in *SLC20A2*. Later, cognitive deficits, progressive hyperkinesias and calcifications in the F13 family were documented; CSF-NfL was elevated, but oxysterol levels were normal in all tested patients indicating that the BBB was intact (**Paper V**). One patient harboring the R467X mutation in *SLC20A2* and affected by ataxia, dementia, and progressive brain calcifications is described (**Paper VI**). *SLC20A2* encodes sodium-dependent phosphate transporter 2. As in *SLC20A2* knockout mouse models, the level of phosphate in her CSF was elevated as was her CSF-NfL. In both the F13 family and the carrier of *SLC20A2* mutation a coregistration was employed to evaluate the progression of calcification.

*GBA1* mutations and variants are risk factors for Parkinson's disease (PD) and other types of parkinsonism. The *GBA1* gene is mutated in connection with Gaucher disease (GD). A GD1 cohort (n=13) and a GD3 cohort (n=12) were examined. In the GD1 cohort two PD cases were identified but none in the GD3 cohort. Abnormal DAT scan was found in 1 GD3 patient and hyposmia was present in 44%. Six GD3 patients have lived beyond 40 years of age. Dystonia was documented as a novel feature in GD3. Neither group had detectable neurological progression during 3 years (**Paper VII**).



## LIST OF PUBLICATIONS

### **I. Genotype-phenotype analysis in inherited prion disease with eight octapeptide repeat**

**insertional mutation.** Paucar M, Xiang F, Moore R, Walker RH, Winnberg E and Svenningsson P. Prion 2013; 7(6):501-10.

**II. Expanding the AOA4 phenotype.** Paucar M, Malmgren H, Taylor M, Reynolds J, Svenningsson P, Press, R and Nordgren, A. Neurology Genetics 2016; 2 (1): e49.

**III. Broad clinical spectrum and widespread neurodegeneration associated with spinocerebellar ataxia type 4.** M. Paucar, M. Engvall, C. Söderhäll, M. Skorpil, P. Fazio, K. Lagerstedt-Robinson, G. Solders, T. Skoog, C. X. Zhang, C. Freyer, A. Wredenberg, M. Angeria, A. Varrone, I. Nennesmo, M. Söderberg, H. Jiao, A. Wedell and P. Svenningsson. Manuscript in preparation.

**IV. *PDGFB* mutations cause brain calcifications in humans and mice.** Keller A, Westenberger A, Sobrido MJ, García-Murias M, Domingo A, Sears RL, Lemos RR, Ordoñez-Ugalde A, Nicolas G, da Cunha JE, Rushing EJ, Hugelshofer M, Wurnig MC, Kaech A, Reimann R, Lohmann K, Dobričić V, Carracedo A, Petrović I, Miyasaki JM, Abakumova I, Mäe MA, Raschperger E, Zatz M, Zschiedrich K, Klepper J, Spiteri E, Prieto JM, Navas I, Preuss M, Dering C, Janković M, Paucar M, Svenningsson P, Saliminejad K, Khorshid HR, Novaković I, Aguzzi A, Boss A, Le Ber I, Defer G, Hannequin D, Kostić VS, Campion D, Geschwind DH, Coppola G, Betsholtz C, Klein C, Oliveira JR. Nature Genetics 2013; 45(9):1077-82.

**V. Progressive brain calcifications and signs in a family with the L9R mutation in the *PDGFB* gene.** Paucar M, Almqvist H, Saeed A, Bergendal G, Ygge J, Holmin S, Björkhem I, Svenningsson P. Neurology Genetics 2016; 2(4): e84.

**VI. Ataxia and increased cerebrospinal fluid phosphate associated with a mutation in the *SLC20A2* gene.** Paucar M, Almqvist H, Jelic V, Hagman G, Jörneskog G, Holmin, S, Björkhem I and Svenningsson, P. Accepted for publication in Journal of Neurological Sciences, February 3<sup>rd</sup>, 2017.

**VII. Novel hyperkinetic dystonia-like manifestation and neurological disease course of Swedish Gaucher patients.** Machaczka M, Paucar M, Björkvall CK, Smith NJ, Cox TM, Forsgren L, Svenningsson P. Blood Cells Mol Dis 2016, available online October 2016. <http://dx.doi.org.proxy.kib.ki.se/10.1016/j.bcmd.2016.10.011>.

These articles will be referred to in the text by their Roman numerals (I-VII).

## OTHER RELATED PUBLICATIONS NOT INCLUDED IN THIS THESIS

### **PSP-CBS with Dopamine Deficiency in a Female with a *FMRI* Premutation.**

Paucar M, Beniaminov S, Paslawski W, Svenningsson P. *Cerebellum* 2016; 15(5):636-40.

### **POLG-Associated Ataxia Presenting as a Fragile X Tremor/Ataxia Phenocopy Syndrome.**

Paucar M, Engvall M, Gordon L, Tham E, Synofzik M, Svenningsson P. *Cerebellum* 2016; 15(5):632-5.

**Feeding dystonia in chorea-acanthocytosis.** Paucar M, Lindestad PÅ, Walker RH and Svenningsson P. *Neurology* 2015; 85(19):e143-4.

**Concomitant Facioscapulohumeral muscular dystrophy and Parkinsonism mimicking Multiple System Atrophy.** Paucar M, Beniaminov S, Solders G and Svenningsson P. *Movement Disorders Clinical Practice* 2015; 3(2): 194-196.

**Mutations in *XPR1* cause primary familial brain calcification associated with altered phosphate export.** Legati A, Giovannini D, Nicolas G, López-Sánchez U, Quintáns B, Oliveira JR, Sears RL, Ramos EM, Spiteri E, Sobrido MJ, Carracedo Á, Castro-Fernández C, Cubizolle S, Fogel BL, Goizet C, Jen JC, Kirdlarp S, Lang AE, Miedzybrodzka Z, Mitarnun W, Paucar M, Paulson H, Pariente J, Richard AC, Salins NS, Simpson SA, Striano P, Svenningsson P, Tison F, Unni VK, Vanakker O, Wessels MW, Wetchaphanphesat S, Yang M, Boller F, Champion D, Hannequin D, Sitbon M, Geschwind DH, Battini JL, Coppola G. *Nat Genetics* 2015;47(6):579–81.

**Novel *APT*X mutation in a Hispanic subject affected by ataxia with oculomotor apraxia type 1.** Paucar M, Alonso I, Eriksson M, Beniaminov S, Coutinho P and Svenningsson P. *Movement Disorders Clinical Practice* 2015; 2 (1): 90-92.

**7 $\alpha$ -hydroxy-3-oxo-4-cholestenoic acid in cerebrospinal fluid reflects the integrity of the blood-brain barrier.** Saeed A, Floris F, Andersson U, Pikuleva I, Lövgren-Sandblom A, Bjerke M, Paucar M, Wallin A, Svenningsson P and Björkhem I. *Journal of Lipid Research* 2014; 55(2):313-8.

**De novo mutations in ataxin-2 gene and ALS risk;** Laffita-Mesa JM, Rodríguez Pupo JM, Moreno Sera R, Vázquez Mojena Y, Kourí V, Laguna-Salvia L, Martínez-Godales M, Valdevila Figueira JA, Bauer PO, Rodríguez-Labrada R, González Zaldívar Y, Paucar M, Svenningsson P, Velázquez Pérez L. *PLOS One* 2013; 8(8):e70560.

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## LIST OF ABBREVIATIONS

AD	Autosomal dominant
AOA4	Ataxia with oculomotor apraxia type 4
AR	Autosomal recessive
AT	Ataxia-telangiectasia
BBB	Blood brain barrier
CGH	Comparative genomic hybridization
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
EEG	Electroencephalography
EMG	Electromyography
ENeG	Electroneurography
GBA	Glucosylceramidase beta
GD	Gaucher disease
HD	Huntington's disease
HDL	Huntington's disease-like
HDL1	Huntington's disease-like 1
HDL2	Huntington's disease-like 2
HU	Hounsfield units
IHC	Immunohistochemistry
IPD	Inherited prion disease
MCSZ	Microcephaly, early-onset intractable seizures and developmental delay
MLPA	Multiplex ligation-dependent probe amplification
OMA	Oculomotor apraxia
OMIM	Online Mendelian Inheritance in Man
PDGFB	Platelet-derived growth factor beta polypeptide

PDGFRB	Platelet-derived growth factor receptor beta polypeptide
PET	Positron emission tomography
PFBC	Primary familial brain calcification
PNKP	Polynucleotide kinase 3'-phosphatase
PRNP	Prion protein
SCA	Spinocerebellar ataxia
SLC20A2	Sodium-dependent phosphate transporter 2
SNV	Single nucleotide variant
SSBR	Single strand break repair
VUS	Variants of unclear significance
WES	Whole exome sequencing
WGS	Whole genome sequencing
XL	X-linked
XPR1	Xenotropic and polytropic retrovirus receptor 1

## **1. What are movement disorders?**

Movement disorders are classified either as hyperkinesias (excess of movements) or hypokinesias (a paucity of movements) unrelated to spasticity or weakness (1). Parkinsonian syndromes are by far the most common cause of hypokinesias. Other terms for hypokinesias include akinesia (loss of movement), bradykinesia (slowness of movement) and hypokinesia (decreased amplitude of movements) while alternative names for hyperkinesias are dyskinesias and abnormal involuntary movements. The most common hyperkinesias include ataxia, chorea, dystonia, myoclonus, tics and tremor (1) (Table 1). The term ataxia, derived from the Greek word for chaos, (also known as asynergia and dyssynergia) describes impaired balance and coordination most often as the result of cerebellar impairment. Chorea, from the Greek word for dance, consists of random involuntary, rapid, irregular and non-sustained movements. Dystonia are sustained and repetitive movements involving agonist and antagonist muscles and leading to abnormal postures. Myoclonic jerks are brief, shock-like involuntary movements caused by muscular contractions (positive myoclonus) or inhibitions (negative myoclonus). Tics are abnormal movements or sounds usually preceded by a sensory urge. Tremor is an oscillatory and rhythmic movement caused by alternating or simultaneous contractions of agonist and antagonist muscles, the location, amplitude and rate of which varies (1)

## **2. The choreic patient**

Assessing the etiology of chorea involves its presentation (insidious or subacute), age of onset (AO), family history and history of exposures, as well as a thorough physical exam. Although family history may provide some clues its absence does not exclude a genetic disease. Associated features and diagnostic signs are searched for employing a variety of ancillary tests including a wide range of laboratory analyses, blood smears, neuroimaging and/or neurophysiological exams (2, 3).

Huntingtonism can be acquired or familial, and chorea is often the side-effect of a drug or caused by structural lesions in the basal ganglia. For instance, patients with Parkinson's disease (PD) develop dyskinesias after long-term treatment with levodopa (L-DOPA) and tardive dyskinesia is a complication observed in patients treated with neuroleptics. Stroke or other lesions in the striatum, brain calcifications (either primary or secondary), complications associated with metabolic disorders (e.g., non-ketotic hyperglycemia) and autoimmune/parainfectious diseases are other causes of chorea. Sydenham's chorea is

common in children, while paraneoplastic chorea is in general rare. Etiological diagnosis is crucial for the proper clinical management, prognosis and, for familial cases, genetic counselling. The etiology of acquired chorea and its work-up have been reviewed anywhere else (2–4).

**Table 1. Summary of Movement Disorders**

<b>Hyperkinesias</b>	<b>Hypokinesias</b>
Akathisia Ataxia Athetosis Ballism Chorea Dystonia Hemifacial spasm Hyperkplexia Hypnogenic dyskinesias Jumping stumps Moving toes and fingers Myoclonus Myokymia Myorhythmia Paroxysmal dyskinesias Periodic movements in sleep REM sleep behavior disorder Restless legs Stereotypy Tics Tremor	Apraxia Blocking tics Cataplexy Catatonia Freezing of gait/ Hesitant gait Hypothyroid slowness Rigidity Stiff muscles

Adapted from Fahn S, Jankovic J and Hallett M. Principles and Practice of Movement Disorders, 2<sup>nd</sup> edition, Elsevier Health Sciences; 2011.

### **3. Huntington's disease (HD)**

The nine familial neurodegenerative disorders referred to collectively as polyglutamine diseases are caused by expansions of the cytosine-adenine-guanine (CAG) sequence, which encodes glutamine (Q), in exons of various genes (5) (Table 2). All but one of these disorders is inherited in an autosomal dominant (AD) manner. The polyQ disorders, of which the most common is Huntington's disease, share traits such as adult-onset, anticipation and certain neuropathological abnormalities. The most common disorder in the polyQ group is

Huntington's disease (HD). SCA12 is another disease associated with CAG expansions, but the expansion is located in the 5'UTR region of the *PPP2R2B* gene (6).

**Table 2: Summary of polyQ disorders and associated genes**

Disease	Pattern of inheritance	Gene	MIM number
Huntington's disease (HD)	AD	<i>HTT</i>	613004
SCA 1	AD	<i>ATXN1</i>	601556
SCA2	AD	<i>ATXN2</i>	601517
SCA3	AD	<i>ATXN3</i>	607047
SCA6	AD	<i>ATXN6</i>	601011
SCA7	AD	<i>ATXN7</i>	607640
SCA17	AD	<i>TBP</i>	600075
DRPLA	AD	<i>ATN1</i>	607462
SBMA or Kennedy's disease	XL	<i>AR</i>	313700

AD: autosomal dominant; DRPLA: dentatorubropallidoluysian atrophy; SBMA: spinal and bulbar muscular atrophy; SCA: spinocerebellar ataxia; XL: X-linked.

The original description provided by young George Huntington in 1872 was succinct, but still regarded as complete (7). Usually presenting between 35 and 44 years of age, HD is characterized by progressive symptoms that include involuntary movements, psychiatric/behavioral symptoms and cognitive decline, often leading to dementia (8). Although the characteristic feature chorea was considered to be the diagnostic gold standard, longitudinal observations have revealed that insidious non-motor features are present early in the course of disease (8–10). Other associated motor features include impaired voluntary control, dystonia, hypokinesia, oculomotor abnormalities, dysarthria and dysphagia (11,12). Affective symptoms (depression and anxiety) and lack of insight are very common, suicidal ideation and suicide risk are elevated. Varying degrees of irritability, impulsiveness, psychotic symptoms, apathy and obsessive behavior occur and can become problematic (8). The cognitive decline is characterized by the early occurrence of executive dysfunction and psychomotor slow-down. Deficits in memory, emotional recognition and attention occur as well and all these symptoms progress into dementia. Other common characteristics include weight loss, sleep disturbances and dysautonomia (8). The rare juvenile form of HD (JHD) also called Westphal variant, is defined by onset before age 20 and representing only 5% of all HD cases, is more severe than the adult form and is associated with other symptoms such as epilepsy, myoclonus and parkinsonian features at earlier stages (8). Life expectancy after

onset is usually 15-18 years (12). Treatment remains symptomatic, but major advances in recent years have paved the way for the ongoing clinical involving gene silencing that was started in 2015 (13,14).

The geographical distribution of HD varies and its frequency thought to be increasing (15). The highest prevalence of HD around the Maracaibo Lake in Venezuela probably reflects a founder effect (16). Although more common than originally thought, especially among Caucasians, this disease is rare in Asian and African populations (15). The United Kingdom is estimated to have about 12 cases per 100 000 inhabitants (17).

A radiological hallmark of HD is the presence of striatal atrophy particularly in the caudate nucleus, as confirmed in the TRACK HD study which found that such atrophy precedes the disease presentation by a decade (18). Such caudate atrophy is not specific for HD, being found in HDL2 and neuroacanthocytosis syndromes as well. The TRACK HD longitudinal assessment of HD also revealed progressive atrophy of the white matter in presymptomatic subjects and in those with early stage disease (18).

### **3.1 The genetics of HD**

The discovery of a marker in the short arm of chromosome 4 in 1983 made genetic testing possible for the first time (19). Ten years later, the underlying pathological CAG expansion in exon 1 of the *HTT* gene was identified (20). The size of this expansion shows an inverse correlation to AO (21). Healthy subjects usually have 7-12 CAG repeats and can have as many as 26. The range of intermediate alleles is 27-35 CAG repeats. Penetrance is incomplete with 35- 39 CAG repeats and complete with a larger number. Larger CAG repeat expansions are associated with greater instability and higher risk for additional expansion in subsequent generations. The size of the CAG expansion determines 44% of the variability in the AO among mutation carriers, with the vast majority of HD patients exhibiting 40-50 CAG repeats which represent (20). A genome-wide association (GWAS) analysis revealed two associated loci with modification of AO (22).

## **4. Huntington's disease-like (HDL)**

HD is by far the most common cause of familial chorea and the most common familial neurodegenerative disease (23), among patients referred for suspected Huntington's disease, 1% lack the pathological nucleotide expansion in *HTT* (24-26) and these conditions are referred to as Huntington's disease-like (HDL) or HD phenocopies (Table 3). Among 285



patients of this kind, only 2.8% were properly diagnosed (23) and the results of several other screenings performed since 2002 have been variable (27–38). Algorithms for the diagnosis of HDL syndromes have been proposed over the years and adapted to recent discoveries and technologies (3, 35). When I began my thesis work in 2011 the most common HDL in Western populations was by far spinocerebellar ataxia type 17 (SCA17). In the largest HDL screening to date including 1712 German and Austrian patients, 9 such cases were detected (27). Previously, an inherited prion disease (IPD) with 8-OPRI was reported in Sweden and later termed Huntington’s disease-like (HDL1) (39, 40). Only one of the HDL screenings identified another case of IPD (23).

**Table 3: Summary of HD phenocopies, *c9orf72* mutations and SCA17 are the most common HDL syndromes in Caucasian populations.**

Disease	Pattern of inheritance	Gene	MIM number
<i>c9orf72</i> mutations	AD	<i>c9orf72</i>	614260
HDL1	AD	<i>PRNP</i>	176640
HDL2	AD	<i>JHP3</i>	605268
SCA1	AD	<i>ATXN1</i>	601556
SCA2	AD	<i>ATXN2</i>	601517
SCA3/MJD	AD	<i>ATXN3</i>	607047
SCA17	AD	<i>TBP</i>	600075
DRPLA	AD	<i>ATN1</i>	607462
BHC	AD	<i>NKK2</i>	600635
Neuroferritinopathy	AD	<i>FTL</i>	134790
ADCY5-related dyskinesia	AD	<i>ADCY5</i>	600293
McLeod syndrome	XL	<i>XK</i>	314850
Chorea-acanthocytosis	AR	<i>VPS13</i>	605978

AD: autosomal dominant, AR: autosomal recessive, BHC: benign hereditary chorea, DRPLA: dentatorubropallidolusian atrophy, HDL: Huntington’s disease like, SCA: spinocerebellar ataxia, XL: X-linked.

Other important AD HDLs include Huntington’s disease-like 2 (HDL2) and *c9orf72* mutations. The former afflicts predominantly patients of African ancestry (41–43) whereas a screening of 514 English patients with HDL identified pathological nucleotide expansions in *c9orf72* in 10 patients (1.95%) making this the second most common cause of familial chorea

after HD (35). Two much smaller screenings appear to confirm that *c9orf72* mutations are a common cause of HDL (38, 44). The clinical presentations of HD and other polyQ disorders, in particular spinocerebellar ataxia type3/Machado Joseph disease (SCA3/MJD) and dentatorubropallidoluysian atrophy (DRPLA) overlap to some degree. Sometimes chorea is associated with SCA1 and SCA2 (3) although two HDL screenings found no SCA1, SCA2, SCA3 or DRPLA cases (23,34).

#### **4.1 *c9orf72* mutations**

Over 60 hexanucleotide GGGGCC expansions in the first intron of the *c9orf72* gene are associated with a spectrum of AD frontotemporal dementia and/or amyotrophic lateral sclerosis (FTD/ALS) (45). Such mutations have reduced penetrance (46), and constitute the most common cause of familial ALS (25%) as well as accounting for 12% of familial FTD. The mean AO among the 10 HDL patients associated with *c9orf72* mutations was 42.7 years (range 8-60 years). Their clinical presentation involved predominant movement disorders, cognitive decline and early psychiatric symptoms. Rigidity and bradykinesia were more common than chorea, with 40% displaying upper motor neuron signs. Six of these demonstrated impaired memory and executive functions, while general brain atrophy was evident in 4 of 8 (35). Atypical parkinsonism has also been attributed to *c9orf72* mutations (47).

#### **4.2 Spinocerebellar ataxia type 17 (SCA17)**

Although SCA17, caused by a pathological CAG expansion in the gene encoding the TATA-binding protein, (*TBP*) was initially described in Japan, this disease is panethnic (48–50). The phenotype varies widely, including, in addition to ataxia and chorea, other motor abnormalities such as parkinsonism in combination with cognitive decline and/or seizures (51, 52).

#### **4.3 Huntington disease-like 1**

Most inherited prion diseases (IPD) cases are caused by point mutations or mutations in the *PRNP* gene that cause premature termination of transcription. Fewer mutations in this context involve insertions of extra octapeptide repeats (OPRI) with insertion of 4-12 such repeats leading to highly variable phenotypes (53–55). F. Xiang and colleagues reported a Swedish family affected by a HD phenocopy later labelled as Huntington disease-like 1 (HDL1) (39). These patients displayed rapidly progressing dementia and severe psychiatric symptoms, as

well as a variable degree of rigidity, ataxia and chorea. HDL1 is caused by insertion of extra 8-OPRI into the *PRNP* gene (40). Insertions of 6-OPRIs carried by a large English family have been reported to result in an HDL phenotype (23).

#### **4.4 Other forms of HDL**

Other forms of HDL include Huntington disease-like 2 (HDL2). The mutation underlying HDL2, originally described in an African American family, involves CTG/CAG repeat expansions in the junctophilin gene (*JPH3*) (41, 56). To date, only two patients of non-African ancestry have been diagnosed with this disease (23, 57). As in the HD cases, the length of the expansion is inversely correlated with the AO. HDL2 accounts for 1% of HDL cases in North America (30). The similarities between its clinical and radiological features and those of HD make it difficult to distinguish between these two (41).

Benign hereditary chorea (BHC), *ADCY5*-related dyskinesia, neuroferritinopathy and paroxysmal dyskinesias are other AD HDL. Symptom onset of BHC usually occurs during childhood and spontaneous amelioration over time is common. BHC is also associated with a broad spectrum of neurological symptoms including ataxia and dystonia, as well as non-neurological features such as hypothyroidism and pulmonary symptoms (58). Mutations in the *ADCY5* gene are associated with complex phenotypes that include in addition to hyperkinesias, delay of motor milestones and exacerbations of other motor features. Some patients display non-progressive disease, as well as hypotonia and a varying degree of intellectual disability (59–61). Paroxysmal dyskinesias are associated with mutations in the *GLUT1* and *PRRT2* genes. Primary familial brain calcifications (PFBC), a group of AD disorders with variable presentation, are discussed in detail below in section 8.1. Neuroferritinopathy, aceruloplasminemia and other conditions included in the category of neurodegeneration with brain iron accumulation (NBIA) display distinctive radiological features (62, 63).

Autosomal recessive (AR) HDL phenotypes include Wilson disease. Chorea can be one feature of mixed AR movement disorders, such as the ataxia syndromes associated with oculomotor apraxia (OMA). A new disorder of this type, ataxia with oculomotor apraxia type 4 (AOA4), has been associated with dystonia (64), see also section 6.1 below. Certain experts group Friedreich ataxia together with the HDL group (3, 23). Recently, a homozygous mutation in *GPR88* was described in siblings with a complex syndrome with chorea (65). The most important neuroacanthocytosis syndromes are AR chorea-acanthocytosis and the X-

linked (XL) McLeod syndrome (MLS). Other XL conditions presenting with hyperkinesias include Lesch-Nyhan syndrome, Lubag disease and Rett syndrome. Chorea occurs in connection with mitochondrial disorders like mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) and Leigh syndrome among others (3). In addition to GLUT1 deficiency, the metabolic conditions associated with chorea include phenylketonuria, glutaric acidemia type I, methylglutaconic aciduria type III and certain aminoacidopathies (3). Additional novel and usually sporadic syndromes are associated with mutations in *PDE10A*, *RNF216*, *GNAO1*, *FOXG1* and *SCN8A* (66).

## 5. Prion diseases

The rare and fatal prion diseases affect mammals only. Establishment of prions (**p**roteinaceous **i**nfectious particle) initially as the cause of scrapie and later of severe neurodegenerative disease in humans, has had far-reaching repercussions (67), especially in the light of reports suggesting that  $\alpha$ -synuclein, the protein that aggregates in Parkinson's disease, spreads and induces protein aggregation in the brain via a *prion-like* mechanism (68). A similar mechanism has been proposed and debated for *huntingtin*, tau and superoxide dismutase among other proteins that undergo abnormal aggregation (69).

The “prion only hypothesis of disease” states that an abnormal prion protein ( $\text{PrP}^{\text{Sc}}$ ) replicates itself by inducing conformational changes in copies of the native prion protein ( $\text{PrP}^{\text{C}}$ ), changes that lead to spongiform encephalopathy, the pathological hallmark of prion diseases. Accordingly, prion diseases are also known as transmissible spongiform encephalopathies (TSEs). The outbreak of bovine spongiform encephalopathy (BSE), referred to as ‘Mad Cow Disease’ by the media, in the United Kingdom in the 1990's had a major impact on the national economy and public health. A novel prion disease, called variant Creutzfeldt-Jakob disease (vCJD) and affecting young individuals emerged in the United Kingdom and was shown to be caused by the BSE prion strain (70).

Molecular classification of prion diseases is based on a combination of prion protein strain ( $\text{PrP}$ ) and genotype (71). Assessment of neuropathology and transmission are also central aspects of the characterization of prion diseases. Although transmission can be investigated by inoculating brain homogenates into transgenic mice expressing the human *PRNP* gene, it should be noted that not all human prion strains are transmissible (72). Human prion diseases are also classified as sporadic, acquired, or familial.

## 5.1 Sporadic prion diseases

This subgroup of human prion diseases is represented by sporadic Creutzfeldt-Jakob disease (sCJD), which with its prevalence of 1-2 cases per million, accounts for 85% of all cases of prion disease. The main feature of sCJD, which usually affects elderly people, is a rapidly progressing dementia in association with extrapyramidal symptoms, ataxia, behavioral and/or visual disturbances (73).

## 5.2 Acquired prion diseases

In addition to vCJD which is mentioned above, this subgroup includes *kuru*, as well as iatrogenic CJD (iCJD). *Kuru* afflicted the Fore people of eastern New Guinea and was caused by the ritual ingestion of the organs of dead relatives (74). iCJD is acquired through contaminated surgical instruments, hormone extracts from the pituitary glands of cadavers and through meningeal and corneal transplantations.

## 5.3 Familial prion diseases

10-15% of all prion diseases are caused by mutations in the prion gene (*PRNP*) located on the short arm of chromosome 20 (75). These mutations exhibit an AD pattern of inheritance and are associated with a wide range of phenotypes, the most common being familial CJD (fCJD), followed by Gertsmann-Sträussler-Scheinker syndrome (GSS) and familial fatal insomnia (FFI). The symptoms of GSS include ataxia, cognitive decline and extrapyramidal disorders. The FFI phenotype involves dementia, insomnia and dysautonomia. The clinical manifestations of a new familial prion disease described by S. Mead et al in 2013 and associated with cerebral amyloid angiopathy include chronic diarrhea, autonomic failure, polyneuropathy, cognitive decline and epilepsy (76). P102L, A117V, D178N, and E200K account for more than 50% of all *PRNP* mutations (77).

### 5.3.1 Polymorphism in codon 129 of the *PRNP* gene

Polymorphism in codon 129 of the *PRNP* gene, which encodes either methionine (M) or valine (V), demonstrates a remarkably strong association with prion diseases. Among Western populations 38% carry MM, 51% MV and 11% VV (78). This polymorphism influences not only susceptibility to prion disease, but also the incubation period prior to the development of acquired prion disease and the clinical phenotype associated with all forms of prion disease

(78,79). For instance, as many as 80-90% of patients with sCJD are homozygous at codon 129. In addition, all cases of vCJD definitively diagnosed so far except one have had the MM genotype (80). Early onset in *kuru* among homozygotes is probably due to a predominantly shorter incubation time, while onset at a later age was more common among heterozygotes (81). This also appears to be the case for iCJD and has been proposed for vCJD as well (80,82). In general, homozygosity is associated with earlier AO of familial prion diseases (54). Another modulation occurs with the mutation D178N in *PRNP*, when the mutated allele is 129M the associated phenotype is FFI and fCJD when it is 129V (83).

### 5.3.2 Extra octapeptide repeats insertions (OPRI)

Most cases of IPD are caused by conventional mutations, but additional octapeptide repeats insertions (OPRI) into the *PRNP* gene, also known as base pair insertions (BPIs), account for a minority of mutations. Insertions of additional 2-12 OPRI in the N-terminal region of *PRNP* are pathogenic (53, 55, 84, 85) whereas loss of a single OPRI is considered to be a polymorphism (75). The N-terminal region normally contains one nonapeptide (R1) followed by four octapeptide units (R2-R4) (54).

Only four families afflicted by IPD with 8-OPRIs and with varying phenotypes have been described, two unrelated French families (Che and M-E), one wDutch (family A) and the fourth Swedish. Four of the seven patients in the Swedish family displayed chorea which was initially attributed to HD (39, 40, 86–88) After HD had been ruled out, this presentation was designated *HDL1* (89). At the time F. Xiang and colleagues described this family, three of its affected members were still alive (39). The *HDL1* phenotype is characterized by rapidly progressing cortical dementia, a variety of movement disorders, and manifest psychiatric/behavioral symptoms. It is noteworthy that some patients belonging to the French M-E family exhibited learning disabilities early in life (88) and antisocial behavior was evident in a large English family with 6-OPRI (90). All of the patients in the Swedish 8-OPRI family are now deceased.

When this thesis was being planned, the effect of the polymorphism in codon 129 of *PRNP* gene on the disease phenotype of IPD with 8-OPRI remained to be characterized. The prion strain underlying this mutation is still unknown. Despite a thorough initial description, the prion-oriented neuropathology of the Swedish 8-OPRI family has not been examined. Varying degrees of cell loss, spongiosis, and astrocytosis in different areas of the brain have

been described in IPD with 8-OPRI (39, 86, 87). In addition PrP plaques were detected in the cerebellum and, to a lesser degree, in the striatum, temporal and parahippocampal cortex (87, 88, 91, 92). Using brain homogenate from one patient of the Che family, transmission to a monkey was achieved (86) although not with a similar preparation from the M-E family (88). Transmission studies involving transgenic mice remain to be performed.

## **6. Ataxia with oculomotor apraxia (AOA)**

Autosomal recessive cerebellar ataxias (ARCA) with oculomotor apraxia are complex and incurable syndromes commonly associated with mutations in genes encoding proteins involved in DNA repair which usually debut early in life (93). The first such disease to be described was ataxia telangiectasia (A-T), which is caused by mutations in the *ATM* gene (94). The A-T phenotype involves of progressive and disabling axial ataxia, peripheral neuropathy, choreoathetosis and variable intellectual disability. Later AO, dystonia and slower progression characterize variant AT in which some enzyme activity remains (94–97). In addition to the neurological features, a wide range of features such as the presence of telangiectasias, sensitivity to ionizing radiation, immunodeficiency with recurrent infections, and pulmonary symptoms are associated with A-T. There is also a remarkable increased in the risk for malignancy, in particular leukemias and lymphomas (94). All these features explain the reduced life expectancy in A-T. Increased levels of alpha-fetoprotein (AFP) are found in the vast majority of A-T patients (94).

Ataxias with oculocephalic dissociation also include ataxia-telangiectasia-like disorder 1 (ATLD1), ataxia-telangiectasia-like disorder 2 (ATLD2), spinocerebellar ataxia with axonal neuropathy (SCAN1), ataxia with oculomotor apraxia type 1 (AOA1), ataxia with oculomotor apraxia type 2 (AOA2), ataxia with oculomotor apraxia type 3 (AOA3) and the recently described ataxia with oculomotor apraxia type 4 (AOA4) (64, 93, 98–101). Elevated levels of AFP but normal serum levels of albumin and cholesterol are the biochemical hallmarks of AT and AOA2. Hypoalbuminemia and hypercholesterolemia with normal AFP are found in patients with AOA1 and AOA3 (101). The main features of these various conditions are summarized in Table 4. Ataxia can occur in association with familial RAD50 deficiency and RNF168 deficiency syndrome, but this is extremely rare (102,103).

**Table 4: Differential diagnosis in ataxia-telangiectasia and related disorders**

Disorder	AT	ATLD1	ATLD2	AOA1	AOA2	AOA3	AOA4	SCAN1
Gene	<i>ATM</i>	<i>MRE11A</i>	<i>PCNA</i>	<i>APTX1</i>	<i>SETX</i>	<i>PIK3R5</i>	<i>PNKP</i>	<i>TDPI</i>
Mean age of onset (years)	< 5	2	Childhood	4.3	13	15.6	4.3	13.7
Ataxia	Y	Y	Y	Y	Y	Y	Y	Y
OMA	Y	Y	N	Y	Y	Y	Y	N
Intellectual disability	Y	N	Y	Y	N	N.A.	Y	N
Dystonia	Y	Y	N	Y	Y	N	Y	N
Chorea	Y	Y	N	Y	Y	N	Y	N
Peripheral neuropathy	Y	Y	N.A.	Y	Y	Y	Y	Y
Other features	Variant AT can lack ataxia	Myoclonus	Hearing loss Short stature Photophobia	Severe course	EPS are milder and less common than in AOA1 and AOA4	Only one family	Severe course	Only one family Pes cavus Seizures
Telangiectasias	Y	N	Y	N	N	N	N	N
Immunodeficiency	Y	N	N	N	N	N	N	N
Increased malignancy risk	Y	N	Y	N	N	N	N	N
Obesity	N	N	N	N	N	N	Y	N
Cerebellar atrophy	Y	Y	Y	Y	Y	Y	Y	Y
AFP	Elevated	N	N	N	Elevated	Elevated	Elevated	N
Albumin	Normal	Normal	N.A.	Low	Normal	Normal	Low levels	Low levels
Cholesterol	Normal	Normal	N.A.	Elevated	Normal	Normal	Elevated	Elevated

All genes in this table except *PIK3R5* code for proteins involved in DNA repair. AOA1-4: ataxia with oculomotor apraxia1-4, A-T: ataxia telangiectasia, ATLD1/2: ataxia-telangiectasia-like disorder 1/2, EPS: extrapyramidal symptoms, SCAN1: ataxia with oculomotor apraxia type 1, Y: yes, N: no, N.A.: not assessed.

**References:** 1. Le Ber I, Dürr A, Brice A. Autosomal recessive cerebellar ataxias with oculomotor apraxia. *Handb Clin Neurol.* 2012;103:333–41. 2. Baple EL, Chambers H, Cross HE, *et al.* Hypomorphic PCNA mutation underlies a human DNA repair disorder. *J Clin Invest.* 2014 Jul;124(7):3137–46. 3. Coutinho P, Barbot C. Ataxia with Oculomotor Apraxia Type 1. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJ, *et al.*, editors. *GeneReviews*(®) [Internet]. Seattle (WA): University of Washington, Seattle; 1993 [cited 2017 Feb 1]. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK1456/> 4. Bras J, Alonso I, Barbot C, *et al.* Mutations in PNKP cause recessive ataxia with oculomotor apraxia type 4. *Am J Hum Genet.* 2015 Mar 5;96(3):474–9. 5. Paucar M, Malmgren H, Taylor M, *et al.* Expanding the ataxia with oculomotor apraxia type 4 phenotype. *Neurol Genet.* 2016 Feb;2(1):e49.



## 6.1 Ataxia with oculomotor apraxia type 4 (AOA4)

In 2015 a novel syndrome resembling AOA1 and AOA2 was described in 11 patients from 8 Portuguese families. This syndrome is associated with biallelic mutations in the *PNKP* (polynucleotide kinase 3'-phosphatase) gene and was designated ataxia with oculomotor apraxia type 4 (AOA4) (64). The mean AO for this progressive syndrome is 4.3 years and it involves ataxia, prominent peripheral neuropathy, dystonia, abnormal eye movements and varying degrees of cognitive impairment as well as obesity. Neuroimaging of AOA4 patients demonstrates cerebellar atrophy in all cases and brain stem atrophy in a few. Elevated levels of AFP (1.5-4-fold) were present in some, while most demonstrated hypoalbuminemia and hypercholesterolemia. Bras and colleagues have suggested that AOA4 is the most common form of AOA in Portugal (64).

Initially, *PNKP* mutations were discovered to be associated with the severe, but non-progressive syndrome referred to as microcephaly, early-onset, intractable seizures and developmental delay (MCSZ) (104). Concomitant MCSZ and hearing loss were also reported in a child harboring mutations in both *PNKP* and *PCDH15* (105). Another MCSZ patient was also affected by short stature and dysmorphism (106). Cerebellar atrophy is also evident in the majority of patients with MCSZ, but in contrast to those with AOA4 can also display brain atrophy, a simplified gyral pattern, thinning of the corpus callosum, enlargement of the ventricles and reduction of white matter (104). The homozygous *PNKP* variant c.58G>A has been reported to be associated with epileptic encephalopathy, although this variant is probably benign (64,107). Patients with MCSZ exhibit an approximately ~10-fold reduction in the levels of PNKP protein in their lymphocytes as well as impaired repair of chromosomal DNA strandbreaks. Moreover, at least three such mutations reduce the stability of the PNKP protein and obliterate kinase activity *in vitro* (108).

## 7. Spinocerebellar ataxias (SCA)

Spinocerebellar ataxias (SCA) are highly heterogeneous autosomal dominant (AD) diseases numbered in order of disease discovery (SCA1-SCA43). Pathological CAG expansions in exons of different genes (polyQ SCA) represent the majority (~60%) of all SCA cases. One of these polyQ SCA, spinocerebellar ataxia type 3/Machado Joseph Disease (SCA3/MJD), is the most common SCA subtype globally. Until recently, SCA7 was considered the most common subtype in Sweden (109) however unpublished data no longer support this notion (Prof. M.

Nordenskjöld). The remaining SCA subtypes are rare and caused by conventional mutations, variations in copy number and non-coding nucleotide expansions. The prevalence of SCA varies in different regions, with some SCAs occurring in very specific populations, e.g. SCA10 described among native populations in the Americas (110–112). Disease onset in SCAs with conventional mutations is usually during childhood and disease progression is slow. In addition, this type of SCAs is usually associated with cerebellar atrophy. Intellectual disability occurs in some specific types of SCA, e.g. SCA13, SCA19 and SCA27 among others. Poly-Q SCA are, in general, more severe diseases and associated with adult-onset, fast progression and atrophy in both the brainstem and cerebellum (110,113)

### **7.1 Spinocerebellar ataxia type 4 (SCA4)**

SCA4, one of the rarest SCA subtypes, was first described in a Scandinavian kindred in the American Midwest and found to be linked to 16q22.1 flanked by the markers D16S514 and D16S512 (114). Adult-onset, slowly progressing ataxia and axonal sensory neuropathy are the hallmarks of this phenotype. This first report claimed that SCA4 was identical to Biemond's ataxia described in a Dutch family, family S, in the 1950's (114,115). Seven years later, Hellenbroich and colleagues described a second SCA4 family in Germany, in which the afflicted had cerebellar atrophy, and also narrowed the candidate region from 6 to a 3.69 cM interval (116). Anticipation was suggested in both the US and German SCA4 families, however a search for pathological trinucleotide expansions on selective genes in the candidate region yielded negative results (116). While Biemond's ataxia has been described as "posterior column damage", only one neuropathological assessment of SCA4 has been published. Unfortunately, this assessment was limited to cerebellum and brainstem and for unclear reasons did not include other regions of the nervous system (117). These investigators found severe loss of Purkinje cells (PC) as well as demyelination of cerebellar and brainstem fiber tracts, and marked neuronal loss in several brainstem nuclei (cranial nerve nuclei, substantia nigra and the inferior olive nucleus) (117).

Linkage to chromosome 16q22.1 in certain Japanese families suggested the possibility of an allelic disorder similar to the Western SCA4. However, the phenotype of this putative Japanese SCA4 was different, including hearing loss in addition to ataxia. Anticipation was also present but peripheral neuropathy was absent (118–120). A variant in the puratrophin-1 (*PLEKHG4*) gene was proposed to be associated with this phenotype (119,121), but this statement was retracted when pathological pentanucleotide expansions in the gene brain-

expressed associated with *NEDD4* (*BEAN1*) gene were identified in these Japanese families and shown to be absent in the Western families with SCA4 (122,123). The Japanese SCA type with linkage to chromosome 16q22.1 was subsequently reclassified as SCA31 and is one of the most common type of SCA in Japan (124). On the other hand, cases of SCA4 are still very rare and the underlying mutation remains to be elucidated.

## **8. Brain calcifications**

The etiology of brain calcifications is extremely broad and usually involves both acquired and idiopathic conditions. The most common acquired causes are mainly endocrine abnormalities (hypo/hyperparathyroidism, pseudohypoparathyroidism), as well as a wide range of infectious agents (toxoplasma, HIV and CMV, among others), trauma, autoimmune diseases, and exposure to certain metals (125,126). In addition, brain calcifications are incidental findings on neuroimaging, particularly on the elderly, ranging from 1-20% (127,128).

The eponym “Fahr’s disease” is taken as synonymous with idiopathic brain calcifications, but the wide use of this term has been questioned for several reasons. First, the clinical presentation with rapidly progressing tetanus and neuropathological findings described by Fahr are indicative of hyperparathyroidism rather than a primary or idiopathic form of brain calcifications. Delacour is credited with being the first to provide an accurate description of a probable case of primary brain calcifications in 1850 (129). Another shortcoming of the eponym Fahr’s disease is that it provides no information concerning the underlying etiology. Over the years, many other terms have been used to denote brain calcifications, as summarized by Manyam and colleagues (130).

### **8.1 Primary familial brain calcifications (PFBC)**

Primary familial brain calcifications (PFBC) are a group of rare AD neurodegenerative disorders with symptom onset usually at an adult age. The clinical presentation varies extensively and most commonly includes a combination of movement disorders, psychiatric symptoms, cognitive decline and headaches/migraine; seizures are unusual. The most common movement disorders are hyperkinesias and Parkinsonism, whereas ataxia is uncommon (131–133). The symmetric calcifications affect the basal ganglia, thalamus, cerebellum, cortex and white matter with the brainstem being less commonly affected.

Even though pseudohypoparathyroidism also exhibits an AD pattern of inheritance, its pleiotropic clinical features are strikingly different from those of PFBC and include childhood

onset, short stature, disabling skeletal abnormalities (due to fibrous dysplasia and fractures) and seizures (134). Brain calcifications also occur in connection with early-onset and severe AR diseases (e.g. Aicardi–Goutières syndrome, Cockayne syndrome among others) or XL diseases (e.g. a rare form of familial hypoparathyroidism); these conditions have been reviewed anywhere else (126). Certain mitochondrial disorders, such as mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS), and myoclonus with epilepsy and ragged red fibers in muscle (MERRF) are also associated with brain calcifications (135,136).

The best way to visualize and assess brain calcifications is by computer tomography (CT) scans, since calcified areas demonstrate varying degrees of intensity on MRI exams (137,138). The degree of calcification is quantified as the Total Calcification Score (TCS) (131), based on visual rating of 10 different brain structures, each of which is assigned a score ranging from 0-5. In cases of PFBC MRI is performed to evaluate potential white matter abnormalities (WMA) and the integrity of infratentorial regions.

In the only article on genetics of familial brain calcifications that had been published at that time, Geschwind and colleagues proposed linkage to chromosome 14q in a family with PFBC (139). In 2001 mutations in the *SLC20A* gene encoding sodium-dependent phosphate transporter 2 (PiT-2) were discovered to cause PFBC (140) followed by the discovery of mutations in the *PDGFRB* gene which encodes the platelet-derived growth factor receptor- $\beta$  (141).

Despite recent advances concerning the etiology of PFBC, information on the natural history of these conditions is still very limited. Also missing are tools that can help predict the probability of PFBC when assessing brain calcifications on incidental CT scans as well as biomarkers.

## **9. GBA-associated parkinsonism**

Idiopathic Parkinson's disease (iPD), the second most common neurodegenerative disorder, is characterized by bradykinesia, tremor, rigidity, postural instability and a broad spectrum of non-motor features such as enhanced risk for cognitive decline/dementia, psychiatric manifestations, REM sleep behavior disorder (RBD), dysautonomia, constipation, pain and hyposmia, among others (142). The neuropathological hallmarks of PD are loss of dopaminergic neurons in the substantia nigra and deposition of  $\alpha$ -synuclein in neurons;  $\alpha$ -

synuclein and ubiquitin are major components of Lewy bodies (LB) and Lewy neurites (LN). Other types of synucleinopathies include dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). Despite overlapping features, the clinical and neuropathological features of PD differ from those of the other synucleinopathies in important respects (143). While PD is chronic, MSA and DLB are examples of atypical parkinsonism which associated with dire prognosis. Other forms of atypical parkinsonism include the tauopathies progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD).

For a long time PD was considered mainly a sporadic disorder, but the discovery that mutations in the *SCNA* gene, which encodes  $\alpha$ -synuclein, cause AD parkinsonism exerted a major impact on research in this field (144). Subsequently, other rare forms of highly penetrant familial PD have also been described (Table 5), although these mutations explain only 3-5% of all the sporadic cases of PD (145). Moreover, a vast body of evidence has established that variants in *LRRK2*, *GBA1* and *SCNA* genes are important risk factors for PD (146,147). These genetic variants are relatively common in certain populations but their penetrance is on the other hand reduced (147).

*GBA1*, which encodes the enzyme  $\beta$ -glucosylceramidase (GCase), is mutated in Gaucher disease (GD), the most common lysosomal storage disorder. GD is an AR, panethnic condition with a prevalence of 1:57000, this prevalence is higher for Ashkenazi Jews (1:855) among whom the carrier frequency of *GBA1* mutations is 1:18 (148,149). Defective GCase in GD leads to accumulation of glucosylceramide in various organs and highly variable manifestations including hepatosplenomegaly, anemia, cytopenias, skeletal abnormalities (pain crisis, fractures and osteonecrosis), occasional pulmonary symptoms and varying neurological features.

Classically, three types of GD have been described based on the presence of neurological symptoms. The most common is type 1 (GD1), long considered to be “non-neuronopathic” (94 % of cases); type 2 (GD2) is the infantile and fatal form (1.1% of cases) and type 3 (GD3) the neuronopathic type (5% of cases). AO for GD2 and GD3 is less than two years, but progression of GD3 is slower allowing survival into the third or fourth decade of life (149). Untreated patients with GD3 survive to the median age of 11.8 years (150). The features of these three forms of GD overlap exist; it is noteworthy that the remaining GCase activity does not correlate with phenotype severity (151). In Norrbotten and Västerbotten provinces in

**Table 5: Summary of known monogenic forms of Parkinson's disease and risk loci**

<b>Locus</b>	<b>Pattern of inheritance</b>	<b>Gene</b>	<b>Gene product</b>	<b>MIM number</b>
Park1/Park4	AD	<i>SCNA</i>	$\alpha$ -synuclein	168601/ 605543
Park2	AR	<i>PARK2</i>	Parkin	602544
Park5	AD	<i>UCHL1</i>	Ubiquitin c terminal hydrolase	613643
Park6	AR	<i>PINK1</i>	Pten-induced putative kinase 1	605909
Park7	AR	<i>PARK7</i>	DJ-1	606324
Park8	AD	<i>LRRK2</i>	Leucine-rich repeat kinase 2	607060
Park9	AR	<i>ATP13A2</i>	Lysosomal type 5 ATPase	606693
Park11	AD	<i>GIGYF2</i>	GRB interacting GYF protein 2	607688
Park13	AD	<i>HTRA2</i>	HTRA serine peptidase 2	610297
Park14	AR	<i>PLA2G6</i>	Phospholipase A2	612953
Park15	AR	<i>FBXO7</i>	F-box only protein 7	260300
Park17	AD	<i>VPS35</i>	Vacuolar protein sorting 35	614203
Park18	AD	<i>EIF4G1</i>	Eukaryotic translation initiation factor 4 gamma 1	614251
Park19	AD	<i>DNAJC16</i>	DNAJ/HSP40 homolog subfamily C member 6	615528
---	Risk locus	<i>SCNA</i>	$\alpha$ -synuclein	168601/ 605543
---	Risk locus	<i>LRRK2</i>	Leucine-rich repeat kinase 2	607060
---	Risk locus	<i>GBA</i>	Glucocerebrosidase	168600

Adapted from Hernandez DG, Reed X, Singleton AB. Genetics in Parkinson disease: Mendelian versus non-Mendelian inheritance. J Neurochem. 2016 Oct;139 Suppl 1:59–74.

northern Sweden, the prevalence of GD3 (1:17500 inhabitants) is unusually high due to a founder effect. This cluster, referred to as the Norrbottnian type of GD3, is associated with homozygosity for L444P in *GBA1* (152–154); its clinical presentation, even within families, is considerably heterogeneous and includes ataxia, spasticity, OMA, squint, seizures, varying degrees of cognitive impairment and kyphosis (150). In other GD3 cohorts, myoclonic epilepsy has been reported (155). Until 1965 splenectomy was performed in all the Norrbottnian GD3 patients but this practice was abandoned when its detrimental effects on progression of neurological symptoms became evident (150,156). Enzyme replacement therapy (ERT) and substrate reduction therapy (SRT) are the current treatment options (157).

Different attempts to estimate the risk that patients with GD and those heterozygous for *GBA1* run of developing PD have given disparate results (158,159). One such study estimated a 20-fold increased risk (160). However, for a large number of Ashkenazi Jews this risk was estimated to be 4.7% and 1.5%, respectively, at 60 years of age; 9.1% and 7.7% at 80 and 0.7% and 2.1% non-carriers (161). These values are lower than those arrived at in the UK and France, which may reflect differences in genotype and other genetic factors (e.g. *LRRK2*) not tested (162,163).

Genotype-phenotype correlations can be made to some degree in GD and GBA-associated parkinsonism. Of the more than 300 *GBA1* mutations identified to date, the most common mutation worldwide in both Jewish and non-Jewish populations is N370S. N370S has been considered a mild mutation but is overrepresented in cases of GD-PD cases, L444P is also common in patients with GBA-associated parkinsonism (158,164). Those homozygous for the N370S mutation tend to display milder symptoms of GD than those who are compound heterozygous. In contrast, homozygosity for L444P is usually associated with a more severe phenotype, either GD2 or GD3; in one cohort L444P accounted for 42% of GD3 cases but the mutation also occurs in GD1 (149,157). In addition to their well-documented association with PD, *GBA1* mutations are also risk factors for DLB and MSA (165,166).

## 10. AIMS

The general aim of this thesis was to characterize a group of familial hyperkinetic diseases and to try to establish genotype-phenotype correlations when possible. The specific aims were:

- To describe the natural history of IPD with 8-OPRIs and to determine the impact of polymorphism in codon 129.
- To elucidate the genotype of a novel AR ataxia syndrome with features overlapping those of AOA1 and AOA2. Secondly, to assess potential genotype-phenotype correlations for AOA4 and MCSZ.
- To perform a comprehensive characterize in detail two Swedish SCA4 families, broadening the neuropathological study and elucidating the underlying mutation. Secondly, to compare Biemond's ataxia with SCA4.
- To characterize the phenotype and genotype of the F13 family with brain calcifications, to describe the natural history of disease in this family and in a patient harboring the R467X mutation in *SLC20A2* and to screen for biomarkers in cerebrospinal fluid (CSF) for these two different forms of primary familial brain calcifications (PFBC).
- To study the course of disease over a three-year period in two Swedish GD cohorts: 13 patients with GD1 and 12 Norrbottnian patients with GD3.



## **11. MATERIALS AND METHODS**

### **11.1 Patients**

The patients included in these studies were referred either to the Department of Neurology or the Department of Human Genetics at the Karolinska University Hospital. For **Paper I** data was collected from medical records and previous publications. For the other papers, the patients were examined in a manner appropriate to their clinical presentation. All the studies included here were approved by the Regional Ethics Committee in Stockholm and oral and written consent were obtained from the patients or next-a-kin.

### **11.2 Methods**

The approaches and rating scales employed in each investigation are described in great detail in Tables 6 and 7. The Total calcification score (TCS) and coregistration were used to assess the size and potential progression of brain calcifications. The Modified Severity Scoring Tool (mSST) was utilized to evaluate patients with Gaucher disease type 3.

#### **11.2.1 Total Calcification Score (TCS)**

The TCS involves rapid and user-friendly visual rating of the following brain structures: caudate nucleus, lentiform nucleus, thalamus, white matter, cortex, cerebellar nuclei, vermis, midbrain, pons and medulla. Each of these structures is assigned a score ranging from 0-5, so that the maximal TCS is 80. Two radiologists must agree on the scoring (131).

**Table 6: Methods employed in the present thesis**

Method	Paper(s)
Polymerase chain reaction (PCR) and Sanger sequencing	I, II, IV and VI
Whole-exome sequencing (WES)	II
Multipoint linkage analysis	III
CSF analysis	III, V and VI
Western blotting for PNKP, GAD65 and calretinin protein	II and III
Brain MRI	II, III, IV, V and VI
Peripheral nerve magnetisation transfer ratio and peripheral nerve diffusion tensor MRI	III
[11C]Flumazenil-PET and [18F]FluoroDeoxyGlucose(FDG)-PET	III
Neuropathological studies and immunohistochemistry (IHC)	III
Brain CT scans	IV, V and VI
Dual-energy computed scans (DECT)	V
40-item University of Pennsylvania Smell Identification Test (UPSIT)	VII
(123)I-N--Fluoropropyl-2b-carbomethoxy-3b-(4-iodophenyl) nortropane ((123)I-FP-CIT)-DaTSCAN (123I-FP-CIT (DaTSCAN®))	VII
Electroneurography (ENeG), electromyography (EMG),	II, III, VI and VII
Quantitative sensory test (QST), variability of RR-interval, skin sudomotor response (SSR), ambulatory polysomnography with Embletta and hearing tests	III

**Table 7: Rating scales and cognitive tests applied in the studies on which this thesis is based.**

<b>Rating scale/tool</b>	<b>Paper(s)</b>
Hospital Anxiety and Depression Scale (HADS)	III, V and VII
Composite Autonomic Symptom Score (COMPASS)	III
Tremor rating scale (TRS)	V and VI
Unified Parkinson's Disease Rating Scale (UPDRS)	V, VI and VII
Scale for the Assessment and Rating of Ataxia (SARA) and Inventory of non-ataxia Symptoms (INAS)	II, III, IV, V and VI
Unified Huntington's disease rating scale (UHDRS)	V and VI
modified Severity Scoring Tool (mSST)	VII
Brief cognitive status: Montreal Cognitive Assessment (MoCA) and Mini Mental State Examination (MMSE)	II, III, V, VI and VII
General intellectual ability (IQ): Ravens Progressive Matrices	V
Evaluation of verbal episodic memory: Rey Auditory Verbal Learning Test RAVLT (RAVLT) and Buschke's Free and Cued Selective Reminding Test (BFCSRT)	V and VI
Visuospatial episodic memory: Rey Osterrieth Complex Figure Test (ROCFT)	V and VI
Assessment of working memory: digit span of the Wechsler Adult Intelligence Scale (WAIS-III) or WAIS-IV	V and VI
Assessment of spatial/visual construction: ROCFT, Copy and Block Design/WAIS	V and VI
Assessment of working memory: digit span of the Wechsler Adult Intelligence Scale (WAIS-III) or WAIS-IV	V and VI
Assessment of spatial/visual construction: ROCFT, Copy and Block Design/WAIS	V and VI
Verbal concept formation: Similarities in WAIS-III	V and VI
Word fluency: Controlled Oral Word Association Test (FAS/COWAT)	V and VI
Picture Naming: Boston Naming Test (BNT)	V and VI
Information processing speed: Symbol Digit Modalities Test (SDMT)	V and VI
Executive function: Trail Making Test, B (TMT)	V and VI
Motor speed: Finger-tapping test (FT), dominant and non-dominant hand	V and VI

### 11.2.2 Coregistration

In **Paper V** coregistration with fusion software was used to optimize comparisons between CT scans. With this approach, 3D regions of interest (ROI) in the reference first CT scan propagate semiautomatically to the follow-up CT scan. The relative and absolute changes in Hounsfield units (HU) were then determined (Integrated Registration, GE AW server 2) and the average of several ROIs used in order to minimize errors. The number of 3D-ROIs utilized varied from 1 to 4 depending on the size of the calcifications. A modified version of this coregistration approach was used in **Paper VI** since an automated optimal 3D match between CT sections was not possible to obtain in this case. Here, two experienced neuroradiologists matched the images as exactly as they could and chose multiple 2D ROIs instead. As with the original method, the HU in these ROIs were determined and the average of these measurements used to calculate the differences. Increases of more than 10 HU were considered significant in both cases.

### 11.2.3 The Modified Severity Scoring Tool (mSST)

One of the scales employed to assess patients with GD3 is the modified Severity Scoring Tool (mSST) which includes 12 domains: gaze palsy, ophthalmology, epilepsy, age at the time of first seizure, cognitive ability, ataxia of gait, cerebellar tremor, pyramidal, extrapyramidal, swallowing difficulties/oral bulbar function, speech, and spinal alignment. The maximal score is 36 points (167).

### 11.2.4 Statistical analysis

Descriptive tests (means  $\pm$  SEM) were used as well as two-tailed paired t tests were used to explore the statistical significance of the difference between two values. A value of  $p < 0.05$  was considered significant. The statistical analysis in **Paper I** was performed with the SPSS package 20 and included graphs, survival curves, linear regression, ANOVA tests and Person's correlation. Most of the cognitive data in **Papers V** and **VI** are presented as z scores and compared to reference values with a z score  $\leq -1.5$  SD indicating a significant deficit (168,169).

## 12. RESULTS AND DISCUSSION

Two hyperkinetic syndromes in a family afflicted by IPD with 8-OPRI and a single case of AOA4 were characterized (170,171).

### 12.1 The impact of polymorphism in codon 129 in IPD with 8-OPRI (Paper I)

The four families with 8-OPRI included a total of 30 patients, 7 of whom were members of the Swedish family. The Swedish family demonstrated significantly longer survival (mean = 15.1 years, SD = 4.3 years) than the other three. An inverse correlation ( $r = -0.642$ ) between early AO (mean = 29.7, SD = 5.4) and long survival was observed, a pattern similar to that described for a large English family with 6-OPRI (172). In general, survival was longer than for cases of sCJD (median = 5 months), iCJD (median = 13 months), vCJD (median = 13 months), fCJD (median = 4 months) or FFI (median = 12 months) (173).

Polymorphism in codon 129 exerted a complex influence on IPD with 8-OPRI. For instance, patients harboring the 129M in the mutated allele had earlier AO, longer survival and earlier age of death than those carrying 129V. Furthermore, those homozygous for 129M had earlier AO than those homozygous for 129V and women had significantly earlier AO and earlier age of death than males (30.7 versus 40.4 years). Regression models demonstrated that as much as 50% of the variability in AO was determined by the combined effects of *PRNP* polymorphism in codon 129 and gender.

Analysis of the pooled clinical features, neuroimaging and biochemical data available in the four 8-OPRI families showed that psychiatric manifestations including personality changes, aggressive behavior, manias and perseverations were the most common symptoms at disease onset. Dementia was a universal feature while ataxia (50%) and parkinsonism (43%) were the most common motor features. Chorea occurred in only 13% of cases, thus, referring to IPD with 8-OPRI as HDL1 may be misleading. The different phenotypic features emphasized by different groups illustrate ascertainment bias.

The course of disease in one of the patients (III:3) of the Swedish 8-OPRI family is described in **Paper I**. We found no evidence of learning disabilities during childhood or antisocial traits such as those reported for family M-E as well as in association with two other *PRNP* mutations (88,90,174). Anticipation has been suggested in IPD with 8-OPRI but the small sample size did not allow a definitive conclusion (88,170). Anticipation was also proposed to occur in connection with the E200K mutation (175), but this was questioned in a larger study

(176). After **Paper I** was published another case of 8-OPRI with a GSS phenotype was reported in the US (177).

Variable cerebral and/or cerebellar atrophy was evident in the four 8-OPRI families; in one case, hyperintensities of the basal ganglia were present. Brain atrophy, particularly in the temporal lobes, was detected in patient III:3 who was shown by HMPAO-SPECT to have reduced blood flow in parietotemporal areas. Similar impaired flow in a female patient from the Dutch 8-OPRI family has been reported (87). Among the 13 of 30 patients for whom EEG data were available 9 exhibited diverse abnormalities and 4 periodic sharp waves complexes (PSWC). None of these four publications report on elevated levels of 14-3-3 in the CSF (39,87,88,178), which along with PSWC are part of the diagnostic criteria for possible sCJD (179).

Genotype-phenotype correlations can be established for some *PRNP* mutations. For instance E200K, the most common *PRNP* mutation, is typically associated with fCJD while P102L is associated with GSS. Other mutations have also been associated with these phenotypes, but only D178N–129M is associated with FFI (54,173). Phenotypes associated with IPD with additional OPRI are highly variable and overlapping. The findings documented in **Paper I** are in line with these observations. The structure of the OPRI expansion in the four 8-OPRI families is unchanged at the amino acid level (88,170). Thus, *PRNP* polymorphisms other than codon 129 and disease modifiers in other genes may explain the striking differences in survival. Transmission experiments could not be performed because the brain tissue of the most recent case (III:3) was fixed in formalin. In the English 6-OPRI family Mead and colleagues demonstrated a pattern of transmission similar to that for sCJD (172). The type of underlying prion strain is still unknown in IPD with 8-OPRI.

This investigation had some limitations. First, *PRNP* polymorphisms other than those in codon 129 were not assessed. The small sample size precluded comparisons between homozygous and heterozygous in codon 129 as well as proper assessment of anticipation. Neither the type of underlying prion strain nor prion-oriented neuropathology was performed due to lack of access to brain tissue. Proper transmission in a transgenic mouse model remains to be performed for IPD with 8-OPRI, but the brain and spinal cord of patient III:3 were maintained in formalin which attenuates transmission (180). Finally, biochemical abnormalities (e.g. levels of CSF-14-3-3) need to be explored in order to identify and validate biomarkers for different types of prion disease.

## 12.2 Novel features of AOA4

The starting point for the present study was a clinical investigation on a 30 year-old Swedish woman born to non-consanguineous parents and afflicted with a complex hyperkinetic syndrome. Trio whole-exome sequencing (WES) (i.e. patient and both non-symptomatic parents) was employed after polyQ SCAs, Friedreichs ataxia and AT were ruled out. Sequencing and MLPA analysis of the *APTX* (mutated in AOA1) and *SETX* (mutated in AOA2) genes prior to mass sequencing were also normal. **Paper II** describes insidious onset of dystonia and chorea at age 5, at age 12 the patient demonstrated ataxia and was diagnosed with pervasive developmental disorder. Areflexia first and finger contractures with distal muscle wasting, loss of proprioception and vibration as well as bilateral foot drop became evident as the disease developed. ENeG and EMG examinations revealed combined sensorimotor demyelinating and axonal polyneuropathy. Dystonia and chorea receded spontaneously with time. At the age of 24 this patient underwent gastric banding for obesity. Since age 25 she can only walk with support and has been in a wheel-chair. Increased saccade latency, broken smooth pursuit and nystagmus were evident upon examination, but OMA was absent. Hypoalbuminemia, hypercholesterolemia and a mildly elevated AFP were present, so treatment with simvastatin and a protein-enriched diet was initiated. Neuroimaging displayed progressive cerebellar, without atrophy in the brain stem. The phenomenology of this case is displayed in a video clip attached to the publication (171).

**Paper II** describes features not previously associated with AOA4, e.g. chorea and slower disease progression. OMA, a universal feature in the Portuguese AOA4 cohort, and variable feature in A-T, AOA1-3 and related disorders was absent (Table 4). Both dystonia and cognitive impairment are also common features in the Portuguese AOA4 cohort. The time to wheelchair in the Swedish patient was longer than in the Portuguese cohort (20 versus 13 years). Obesity described in 30% of all reported cases of AOA4 may provide a diagnostic clue, since this is not commonly seen in other ARCA with oculomotor apraxia. Obesity also occurs in connection with Joubert syndrome type 8, but this disease is far more severe and its radiological features ("molar tooth sign") distinctive (181). Subsequent to the submission of **Paper II**, two additional cases of AOA4 in Brazil and Norway have been reported (182,183). Ataxia and polyneuropathy were present in those patients, but not dystonia or chorea. In the Brazilian patient peripheral neuropathy was predominant, preceding ataxia and cognition was normal (182). The Norwegian patient displayed mild cognitive impairment and developed severe anasarca as the result of hypoalbuminemia. Her neuroimaging revealed widespread

atrophy of the brain, cerebellum and brain stem as well as hyperintensities in the middle cerebellar peduncles (183). In AT, ATM activity is correlated to phenotype severity (97). Whether this also explains the degree of disease severity in cases of AOA4 and MCSZ remains to be determined.

ENeG and EMG examinations revealed demonstrated sensorimotor demyelinating and axonal neuropathy in three cases of AOA4 (171,183,184) and sensorimotor demyelinating neuropathy and axonal neuropathy, respectively, in two other cases (182,184). All of the Portuguese patients had peripheral neuropathy but of which type was not reported (64). One of the authors informed us that they had detected axonal neuropathy in 6 AOA4 patients (personal communication from Dr Barbot). A summary of the *PNKP* mutations and associated phenotypes are summarized in Table 8. The biochemical abnormalities associated with AOA4 include those observed for AOA1 and AO2, i.e. hypocholesterolemia, hypoalbuminemia and elevated AFP. These abnormalities have yet to be reported in MCSZ.

Two new compound heterozygous variants in the kinase domain of the *PNKP* gene, c.1196T>C (p.Leu399Pro) and c.1385G>C (p.Arg462Pro), were identified by WES. These variants, confirmed by Sanger sequencing and predicted to be pathogenic by *in silico* analysis. The level of PNKP protein in the lymphocytes of this patient was low (**Paper II**).

Shen and colleagues have demonstrated not only reduced levels of PNKP, but also impaired DNA repairing in cells isolated from MCSZ patients (104). This impairment has also been demonstrated by chemical induction of apoptosis with chemicals in cell cultures derived from a Brazilian AOA4 patient (182).

To date, 29 cases featuring MCSZ and AOA4 with 14 different *PNKP* mutations have been described, allowing a certain amount of genotype-phenotype correlation. For instance all of the mutations associated with AOA4 are located in the kinase domain of *PNKP*. Only two cases carrying the same homozygous mutation (c.1250\_1266dup) in the kinase region has been described, in one case it was associated with MCSZ and in a second with combined features of MCSZ and AOA4 (104,184). The remaining mutations associated with MCSZ are located in the forkhead and phosphatase domains of the *PNKP* gene (171), suggesting that phosphatase may be more important than the kinase activity during embryogenesis.

As for many other neurological disorders, *PNKP* mutations are associated with a broad spectrum of phenotypes. Only two patients harboring *PNKP* mutations in association with



**Table 8: *PNKP* mutations are associated with non-progressive microcephaly, early-onset of disease, intractable seizures and developmental delay (MCSZ) and the progressive ataxia with oculomotor apraxia type 4 (AOA4).**

Study author (reference)	Nr of cases	Microceph	Dev. del. or intel. disab.	Behav. abn.	Seizures	PNP	Ataxia	Dystonia	Chorea	OMA	Neuroimaging	Obesity	S-AFP	S-Albumin	s-Cholesterol	Western blotting for <i>PNKP</i>
Shen et al (1)	11	Y	Y (11/11)	Hyperact.	Y (11/11)	N	N	N	N	N	Widespread abnormalities <sup>a</sup> BA, CA	N	N.A.	N.A.	N.A.	Reduced levels
Poulton et al (2)	2	Y (2/2)	Y (2/2)	Hyperact.	Y (2/2)	Y (2/2)	Y (2/2)	N	N	N	BA (2/2) CA (2/2) <sup>b</sup>	N	N.A.	N.A.	N.A.	N.A.
Nakashima et al (3)*	1	Y	Y	N	Y	N	N	N	N	N	Widespread abnormalities <sup>a</sup> CA	N	N.A.	N.A.	N.A.	N.A.
Nair et al (4)**	1	Y	Y	N	Y	N.A.	N	N	N	N	BA Agenesis of CC	N	N.A.	N.A.	N.A.	N.A.
Bras et al (5)	11	N	Y (8/11)	N	N	Y (11/11)	Y (11/11)	Y (8/11)	N	Y (11/11)	CA (11/11) BSA (3/11)	Y (4/11)	Elevated (5/11)	Low (6/9)	Elevated (5/9)	N.A.
Pedroso et al (6)	1	N	N	N	N	Y	Y	N	N	N	CA	N	N.A.	N.A.	N.A.	N.A.
Paucar et al (7)	1	N	Y	N	N	Y	Y	Y	Y	N	CA	Y	Mildly elevated	Low	Elevated	Reduced levels
Tzoulis et al (8)	1	N	Y	N	N	Y	Y	N	N	Y	BA, CA and BSA <sup>b,c</sup>	N	Normal	Low	Elevated	NA

An intermediate phenotype combining features of MCSZ and AOA4 has been described by Poulton *et al* (2).<sup>a</sup>The severe radiological abnormalities in cases of MCSZ include brain and cerebellar atrophy, as well as a simplified gyral pattern, thinning of the corpus callosum, enlargement of the ventricles and a reduction of white matter. All patients with AOA4 patients display cerebellar atrophy and other abnormalities are found. <sup>b</sup>Enlarged ventricles (in one of the siblings), <sup>c</sup> Hyperintensities in the medium cerebellar peduncles. \* Nakashima reported a child with concomitant hearing loss due to *PCDH15* mutations. AFP: alpha-fetoprotein; \*\* P. Nair reported a MCSZ patient with short stature and dysmorphism. BA: brain atrophy, BSA: Brain stem atrophy, CA: cerebellar atrophy, CC: corpus callosum, Hyperact: Hyperactivity, Intel. disab. Intellectual disability, Microceph: microcephaly, OMA: oculomotor apraxia, PNP: peripheral neuropathy. NA: not assessed. N: no, Y: yes.

**References:** 1. Shen J, Gilmore EC, Marshall CA et al. Mutations in *PNKP* cause microcephaly, seizures and defects in DNA repair. *Nat Genet.* 2010;42(3):245–9. 2. Poulton C, Oegema R, Heijnsman D et al. Progressive cerebellar atrophy and polyneuropathy: expanding the spectrum of *PNKP* mutations. *Neurogenetics.* 2013;14(1):43–51. 3. Nakashima M, Takano K, Osaka H, et al. Causative novel *PNKP* mutations and concomitant *PCDH15* mutations in a patient with microcephaly with early-onset seizures and developmental delay syndrome and hearing loss. *J Hum Genet.* 2014 Aug;59(8):471–4. 4. Nair P, Hamzeh AR, Mohamed M, Saif F, Tawfiq N, El Halik M, et al. Microcephalic primordial dwarfism in an Emirati patient with *PNKP* mutation. *Am J Med Genet A.* 2016 Aug;170(8):2127–32. 5. Bras J, Alonso I, Barbot C et al. Mutations in *PNKP* cause recessive ataxia with oculomotor apraxia type 4. *Am J Hum Genet.* 2015;96(3):474–9. 6. Pedroso JL, Rocha CRR, Macedo-Souza et al. Mutation in *PNKP* presenting initially as axonal Charcot-Marie-Tooth disease. *Neurol Genet.* 2015 Dec;1(4):e30. 7. Paucar M, Malmgren H, Taylor M et al. Expanding the ataxia with oculomotor apraxia type 4 phenotype. *Neurol Genet.* 2016 Feb;2(1):e49. 8. Tzoulis C, Sztromwasser P, Johansson S et al. *PNKP* Mutations Identified by Whole-Exome Sequencing in a Norwegian Patient with Sporadic Ataxia and Edema. *Cerebellum Lond Engl.* 2017 Feb;16(1):272–5.

ataxia and microcephaly have been described (184). The role of gene modifiers in *PNKP* mutations has not yet been assessed.

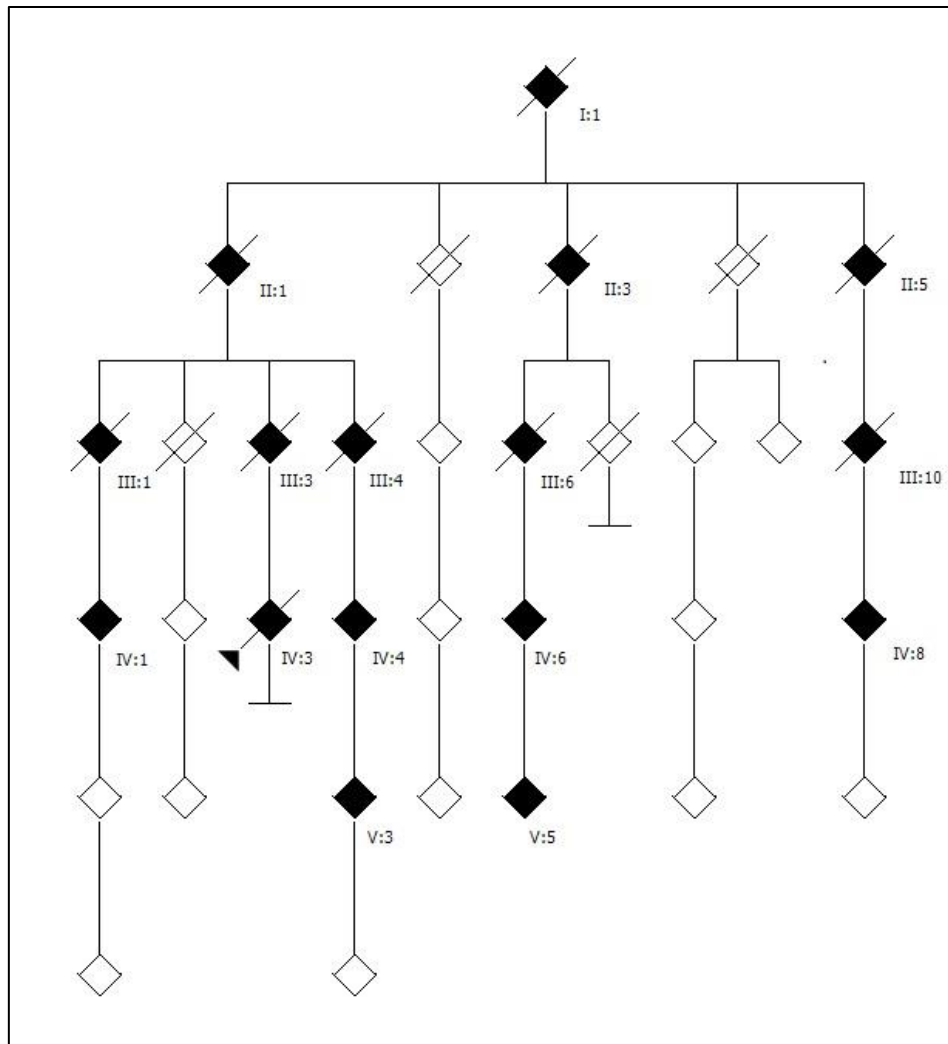
Altogether, the findings documented in **Paper II** strengthen the association between *PNKP* mutations and AOA4. The reduced level of PNKP protein in the lymphocytes of the Swedish patient supports pathogenicity of these *PNKP* variants. However the exact mechanism(s) underlying this disease is still unknown. Shen and colleagues have shown that apoptosis occurs in both neuronal precursors and neurons when *PNKP* is knockdown *in vitro* (104). *PNKP* is crucial for early embryogenesis, since transgenic mice harboring the most common mutation associated with MCSZ (T424Gfs) die early *in utero*. A hypomorphic mouse model of microcephaly exhibits increased apoptosis in the brain (185).

Single strand break repair (SSBR)/base excision repair (BER) is one of a number of pathways for DNA repair (186,187). There is a remarkable selectivity for neurological phenotypes in genetic defects of this pathway (*PNKP*, *APTX* and *TDPI*). However, the matter is complex, since *knockout* mice for *APTX* (mutated in AOA1) and *TDPI* (mutated in SCAN1) lack evident phenotypes (185,188). In addition, no evidence of microcephaly in cases of AOA1 and SCAN1 has been reported to date. Microcephaly does occur in connection with other severe diseases (e.g. Nijmegen breakage syndrome, RAD50 deficiency and RNF168 deficiency) associated with genetic defects in DNA repair pathways other than SSBR (187,189).

One limitation to this study is that apoptosis in lymphocytes challenged with UV and/or chemicals was not evaluated which was done for other *PNKP* mutations (104,182,184). It is unknown whether AOA4 and the other ataxias associated with genetic damage to DNA repair involved enhanced neuronal apoptosis during embryogenesis and/or later in life.

### **12.3 The broad spectrum of symptoms in SCA4 (Paper III)**

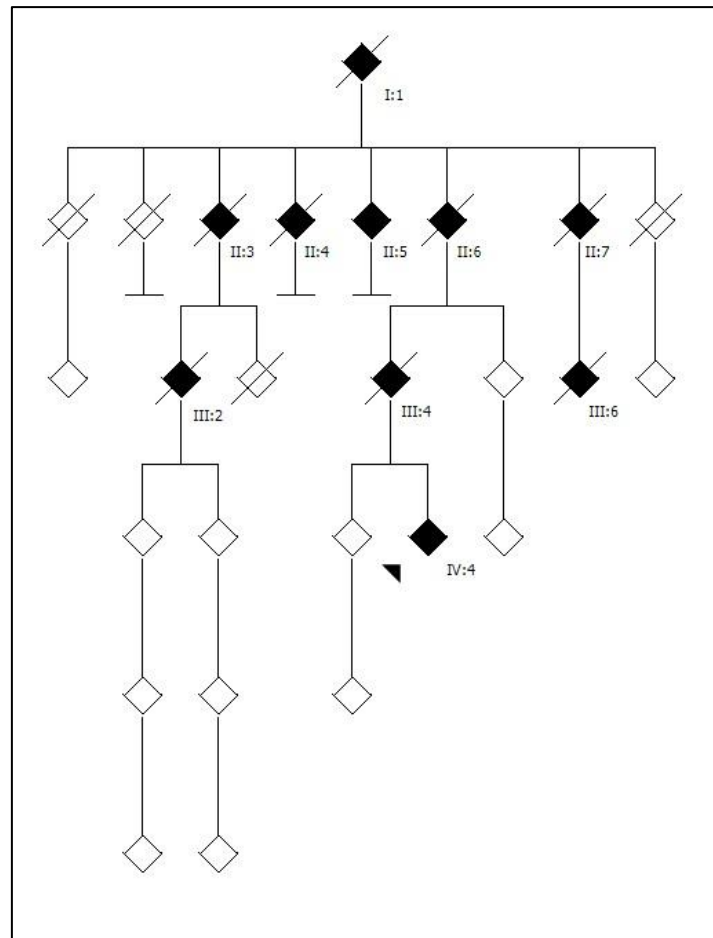
16 members of the first SCA4 family (Figure 1) and 10 of the second one (Figure 2) displayed symptoms. Both families lack the SCA31 mutation. AO was 46.5 years (range 20-60, SD = 10.5) and disease duration was 31.8 years (range 18-55, SD = 11.3) years. Anticipation is again suggested at least in the first family but limited sample size in the previous generations precluded a definitive conclusion.



**Figure 1:** Pedigree of the first Swedish SCA4. Anticipation is suggested in one of the branches of this family. Dysautonomia was a frequent feature in this family.

Varying degrees of dysautonomia were found in all the subjects examined and documented in the other patients. These features included orthostatism, obstipation, abnormal sweating, weight loss, central sleep apnea, incontinence, and erectile dysfunction among others. In two cases dysautonomia preceded ataxia. In contrast to previous reports we found that dysarthria ( $n = 14$ ) and dysphagia ( $n = 10$ ) were common. In addition to abnormal cerebellar eye movements, there were abnormalities associated with brainstem dysfunction (e.g., slow saccades and/or varying degrees of ophthalmoplegia), as well as movement disorders other than ataxia like dystonia, laryngospasm and chorea. Weakness, myokymias and Babinski's sign were evident in some patients and presbycusis present in four patients from both families. Cognitive decline was documented in two patients only, following a stroke and

during the final year of life, respectively. In the latter case neuropathological features compatible with Alzheimer's disease were present.



**Figure 2:** Pedigree of the second Swedish SCA4 family. Dystonia afflicted some of the patients in this family.

All of the patients examined with neurography displayed an axonal sensorimotor neuropathy, and impairment of small fibers was also detected. Peripheral neuropathy is common among cases of SCA in general (190), but universal in SCA4 where dysautonomia is also more common than for other SCAs. These phenotypic features are compared to previous reports in Table 9.

**Table 9: Comparison of the two Swedish SCA4 families to the two families published previously**

Study	Age of onset (mean, years)	Anticipation	Ataxia	Peripheral neuropathy	Dysautonomia	Eye movement abnormalities	Other movement disorders than ataxia	Neuroimaging
Flanigan et al (1)	38.3	Suggested	Y	Y	N	Y	N	N.A.
Hellenbroich (2)	39.3	Suggested	Y	Y	N	Y	N	C.A.
Paucar et al (manuscript)	46.5	Suggested	Y	Y	Y	Y	Y	CA among others*

\* Atrophy in the brainstem and spinal cord was evident in all patients examined. Fractional anisotropy was reduced in the sciatic nerve of three Swedish SCA4 patients. C.A. Cerebellar atrophy; N.A. Not assessed; N: no; Y: yes.

**References:** 1. Flanigan, K., Gardner, K., Alderson, K., Galster, B., Otterud, B., Leppert, M.F., Kaplan, C., Ptáček, L.J., 1996. Autosomal dominant spinocerebellar ataxia with sensory axonal neuropathy (SCA4): clinical description and genetic localization to chromosome 16q22.1. *Am. J. Hum. Genet.* 59, 392–399. 2. Hellenbroich, Y., Bubel, S., Pawlack, H., Opitz, S., Vieregge, P., Schwinger, E., Zühlke, C., 2003. Refinement of the spinocerebellar ataxia type 4 locus in a large German family and exclusion of CAG repeat expansions in this region. *J. Neurol.* 250, 668–671. doi:10.1007/s00415-003-1052-x. 3. Paucar, M. et al. Broad clinical spectrum and widespread neurodegeneration associated with spinocerebellar ataxia type 4. Manuscript.

Neuroimaging revealed variable atrophy in the vermis, pons, medulla, substantia nigra and spinal cord. Fractional anisotropy (FA) in the sciatic nerve was low in patients previously found to have periphery neuropathy. 18F-FDG-PET showed a global reduction in brain metabolism. Reduced binding in the cerebellum, insula, thalamus and in several cortical regions (frontal, parietal, temporal, limbic and occipital) was also observed by 11C-Flumazenil-PET, indicating loss of GABA-ergic neurons. Setting the ROIs manually did not change this reduction in the vermis but made it more apparent in the hypothalamus. The significance of this reduction is unclear, but may be related to the dysautonomia in the examined patients.

Assessment of neuropathology in the index case of the first family and case III:2 from the second revealed mild atrophy of the vermis and mild-to-moderate loss of Purkinje cells (PC) was found in the former, where the number of calretinin-positive cells in the granular layer was also reduced. Expected gliosis was evident in the right external capsule and corona radiata following a stroke at age 76. In the second case, a mild reduction in cortical thickness, widening of the frontal sulci, presence of diffuse neuritic plaques and neurofibrillary tangles in the entorhinal cortex and, to a lesser degree, in the hippocampus were evident. In contrast to the first case, the loss of PC was moderate to severe and proliferation of Bergmann glia was also detected. Both cases demonstrated loss of neurons in the olive nucleus, severe loss of motor neurons in the anterior horn, and degeneration of the posterior columns. Immunohistochemistry (IHC) analysis of the first case revealed intranuclear inclusions, staining for p62 and ubiquitin in the remaining motor neurons of the anterior horn as well as accumulation of cells in the vicinity of the central spinal canal. No obvious neuronal loss was noticed in the dorsal root ganglia (DRG), but a scarcity of fibers in the ventral and dorsal roots was evident. Three cases displayed moderate-to-severe loss of myelinated small and thick fibers in the right suralis nerve, with Wallerian degeneration. In summary, these abnormalities are far more far-reaching than the neuronal loss in the cerebellum and brainstem reported for a German SCA4 patient (117). The only similarities with Biemond's ataxia were degeneration of the posterior columns, mild loss of PC and demyelination of cerebellar fiber tracts (115). In contrast to Biemond's findings and the German SCA4 case, we found no evidence of damage to the trigeminal nuclei. The CSF of two members of the first SCA4 family was analysed and the level of tau was found to be elevated and beta-amyloid reduced in the index case. However, there was no neuropathological evidence of Alzheimer's disease, with normal levels of CSF-NfL in both cases, despite widespread neurodegeneration. In both

cases as well, the level of 5-hydroxyindoleacetic acid (5-HIAA) was normal but that of homovanillic acid (HVA) elevated, giving a high ratio HVA/5-HIAA that may reflect impaired turnover of serotonin. The level of 4-hydroxy-3-methoxyphenyl glycol (HMPG) in the CSF was reduced. The protein levels of GAD65 and calretinin were reduced in the temporal cortex and cerebellum respectively in two SCA4 patients.

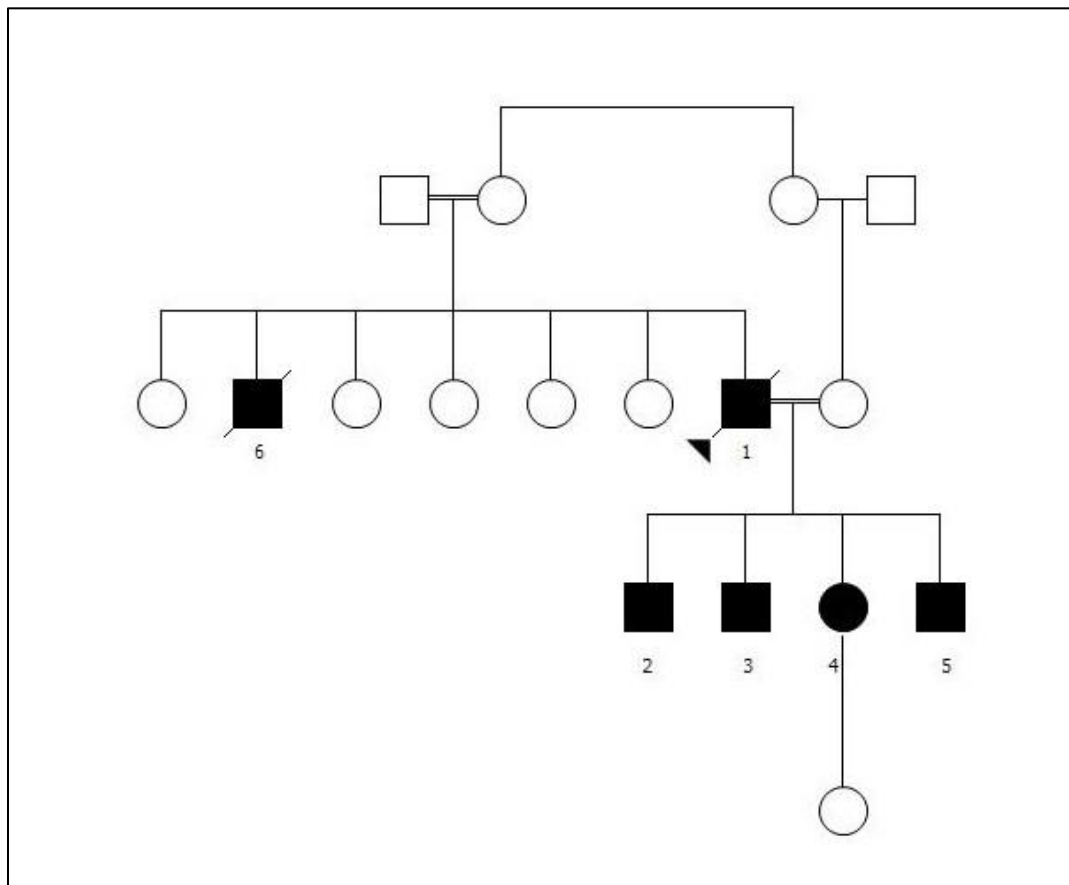
Multipoint analysis confirmed linkage to chromosome 16q22.1 with a maximum LOD score of 3.7 being located in a 3.69 cM (25 Mb) region between D16S3086 and D16S512. The second family shared the same haplotype markers. However, sequencing of this candidate region did not yield clear variants that segregated with disease in either family.

Although, this description expands the phenotypic characterization of SCA4, the underlying mutation remains elusive. Linkage to other chromosome regions than chromosome 16 was not analysed which represents a limitation of this work. With respect to neuropathology, assessment of the GABA neurotransmitter system and correlation to observed PET abnormalities is required.

#### **12.4 Biemond's ataxia appears not to be SCA4**

Two of the four generations of Family S were consanguineous and the patients were affected by sensory and cerebellar ataxia (Figure 3). In contrast to SCA4, the debut of symptoms was subacute in two of these patients who also suffered starvation during World War II. Moreover, the symptoms of a third patient were exacerbated by starvation. Other features uncommon or absent in SCA4 were present in this family were deafness (1), optic atrophy (2, one whom became blind) and chorea (2). In addition three patients did not progress and one recovered spontaneously. Aggressive progression leading to death after 6 and 7 years occurred in the remaining two patients, one of whom was examined post mortem. Biemond found degeneration of the posterior columns, trigeminus nucleus, notorious demyelination and mild loss of Purkinje cells, but there was no evidence of cerebellar atrophy. Lack of progression and recovery occurred in those exposed to starvation. Taken together, these features argue strongly against SCA4. Biemond himself mentioned nutritional deprivation as a trigger of disease but wrote about an "endogenous predisposition" (115). The pedigree of family S appears to be pseudodominant, with some patients likely to have been affected by Strachan's syndrome and others to have suffered from an autosomal recessive disease. The course of disease in the latter is compatible with either a disorder in peroxisome biogenesis or a mitochondrial disorder but the exact etiology of Biemond's ataxia remains obscure. After

the claims made by Flanigan and colleagues, two other contentious descriptions of Biemond's ataxia have been published (191,192).



**Figure 3:** Pedigree of family S as with the same numbers used by Biemond. Notice the consanguinity in two generations. Symptom onset was subacute in patients 2, 3 and 4, who also suffered from starvation during World War II. Symptoms were exacerbated during starvation in patient 2. On the other hand, insidious onset but aggressive course of disease was described in patients 1 and 6, with disease duration of 6 and 7 years respectively. In addition patients 3, 4 and 5 did not progress and one recovered partially spontaneously (Patient 2). **Reference:** Biemond, A., 1954. Radiculo-posterior cord form of spinocerebellar degeneration. Rev. Neurol. (Paris) 91, 3–21.

### 12.5 Mutations in *PDGFB* cause brain calcifications (Paper IV)

The study described in **Paper IV** started as a clinical investigation of a family (referred to hereafter as F13) with autosomal dominant brain calcifications, where the father and three of his children were affected. This family was included in a multicenter cohort investigated by a team lead by Professor Geschwind and Coppola at University of California Los Angeles. In



**Paper IV** a cohort of 6 PFBC families of diverse ethnic backgrounds with a total of 31 patients was characterized. This cohort was screened for secondary causes of brain calcifications, and then for mutations in the *SLC20A2* and *PDGFRB* genes later. This led to the discovery that mutations in *PDGFB* can cause brain calcifications. *PDGFB* encodes platelet-derived growth factor subunit B, the main ligand of *PDGFRB*.

The symptoms documented here included movement disorders (14), migraine (13) and cognitive deficits or psychiatric symptoms (9); some were asymptomatic (5) despite the presence of calcifications. The average AO was 23.9 years. Brain calcifications were located in the basal ganglia, thalamus, white matter and cerebellum. WES was first performed on two families, and later Sanger sequencing of the entire coding regions of *PDGFB* in 30 PFBC families, as well as 22 sporadic cases. Of the six pathogenic *PDGFB* variants identified, the mutation c.26T>G (L9R) was carried by all the patients and none of the healthy controls in family F13 (193).

Since *PDGFB* knockout in mice leads to early death, a hypomorphic *PDGFB*<sup>ret/ret</sup> mouse model was employed to obtain functional evidence of pathogenicity. This model is well-known for its severe glomerular and retinal defects, but normal life-span due to pericyte loss and increased permeability of the blood-brain barrier (BBB) (194,195). In **paper IV** progressive brain calcifications, in the basal forebrain, thalamus, midbrain and pons containing calcium phosphate, were demonstrated in the *PDGFB*<sup>ret/ret</sup> mice. Previous investigations have shown that the gene product of the hypomorphic *PDGFB*<sup>ret/ret</sup> model is truncated in these animals (194). The *PDGFB* gene contains 7 exons and its product is a dimer. The L9R mutation in the signal peptide (exon 1) of *PDGFB* is predicted to disrupt its function. Subsequently, Vanlandewijck and colleagues (196) found the protein levels of *PDGFB* were undetectable when expressed *in vitro*. In addition, this mutated protein failed to activate its receptor (*PDGFRB*). **Paper IV** suggested a correlation between the degree of pericyte loss in the brain and calcifications. However, it was later demonstrated that the calcified areas have a more intact BBB than non-calcified areas (196).

## 12.6 Progressive symptoms and brain calcifications associated with a mutation in *PDGFB* (Paper V)

In **paper V** the F13 family was followed-up and their brain calcifications and signs found to be progressive. The clinical features of four patients were documented by video recordings. The mean clinical follow-up was 5.5 years and time that elapsed between two successive

brain CT scans 4.8 years. All the four patients were diagnosed with migraine with aura and displayed mild eye movement abnormalities. The index case (III:1), was diagnosed with anomia, cognitive deficits and has since developed posturing and chorea. One of her siblings (III:2) has a history of mixed substance abuse and developed chorea despite the absence of radiological progression. The youngest sibling (III:3) exhibited chorea, mild postural tremor and cognitive deficits. Their father (II:3) has developed mild motor features with impaired tandem gait.

Radiological examination revealed that patient II:3 has calcifications in the lentiform nucleus and white matter only. The three patients in generation III have calcifications in these regions as well as in the caudate nucleus, thalamus and dentate nucleus (Figure 4). The TCS indicated progression in two cases (III:1 and III:3) and the coregistration procedure progression in three (II:3, III:1 and III:3).

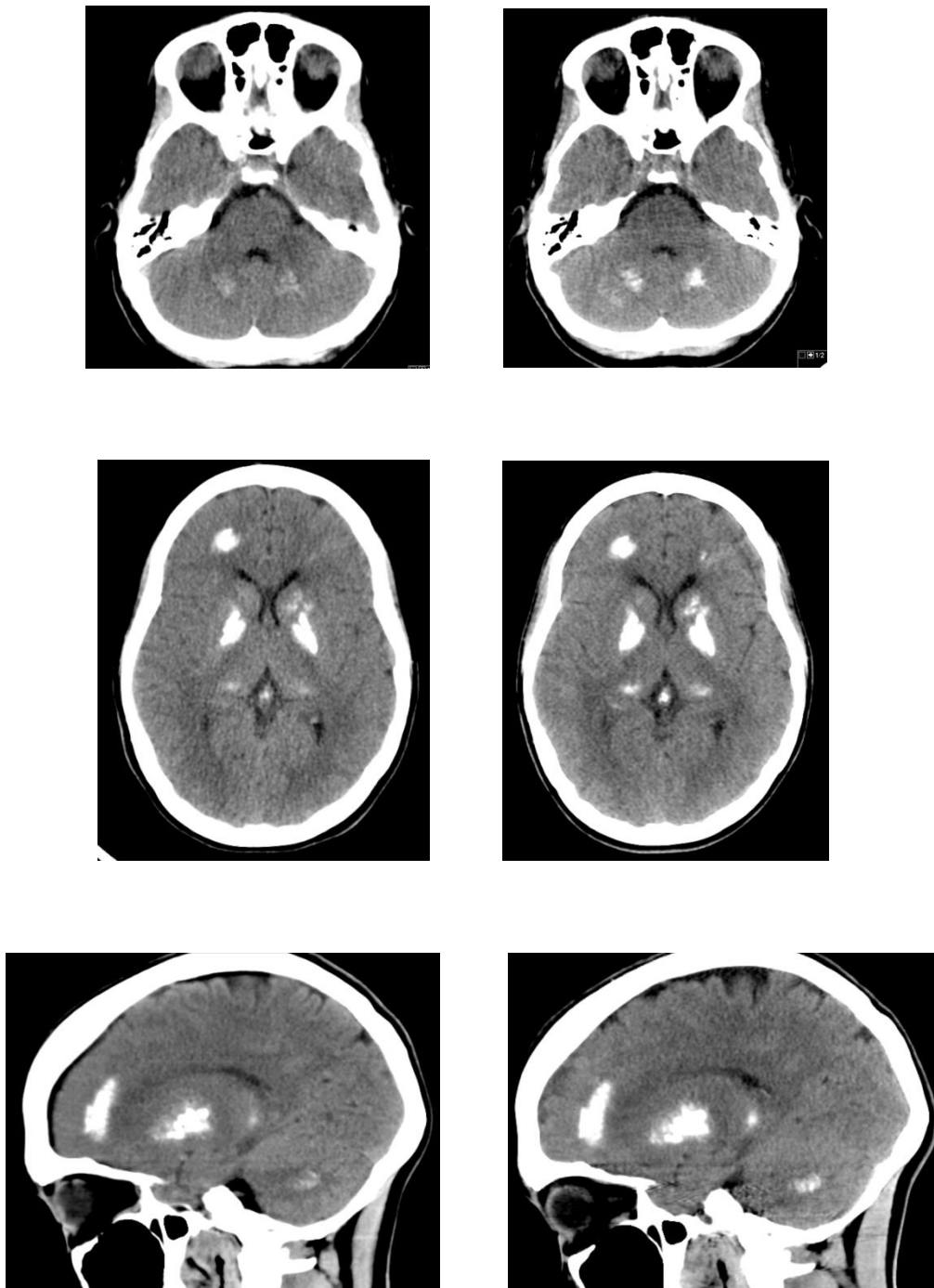
Saeed and colleagues demonstrated that the oxysterol 24(S)-hydroxycholesterol (24S-OHC) is low in the brain but increased in the circulation of the hypomorphic *PDGFB*<sup>ret/ret</sup> mouse model which is compatible with impaired BBB integrity (197). In contrast, oxysterol levels were normal in both the plasma and CSF of three patients from generation III. At the same time, the level of CSF-neurofilament light chain (CSF-NfL) was mildly elevated in the patient with clear cognitive impairment (III:3). The index case (III:2) had a mildly elevated albumin CSF/serum ratio.

**Paper V** provides documents clear evidence of clinical and radiological progression for *PDGFB*-associated PFBC. Two modalities of coregistration, with greater sensitivity than TCS, detected the progression of calcifications, although the former are more time-consuming (**Papers V and VI**). Another advantage with coregistration is overcoming the ceiling effect of TCS. Calcifications in PFBC have been assumed to be progressive on the basis of distribution of TCS at different ages, but only in one genetically confirmed case has radiological progression been reported (198).

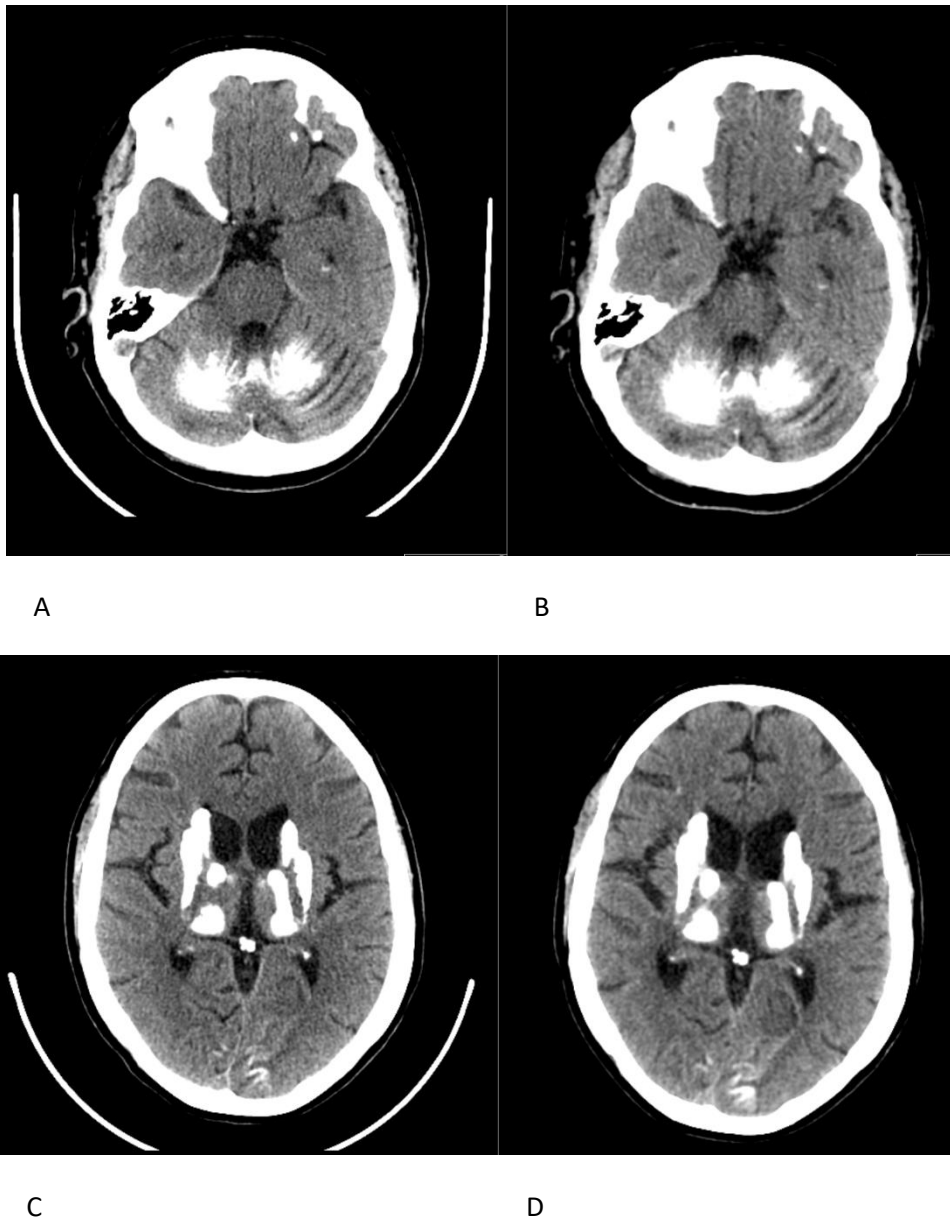
### **12.7 Elevated levels of phosphate in the CSF of a patient with a mutation in *SLC20A2* (Paper VI)**

In **Paper VI** a Swedish patient with impaired balance, cognition and brain calcifications (Figure 5) was found to harbor the c.1399C>T (R467X) mutation in the *SLC20A2* gene. The same mutation was reported previously in a Japanese male affected by paroxysmal kinesigenic dyskinesias (PKD) (199). The radiological penetrance in the Japanese case was reduced, but this feature was not possible to evaluate in the Swedish patient who had a

medical history of type 1 diabetes (T1DM) with complications. AO was 42, her phenotype was assessed and documented by video recordings.



**Figure 4:** Neuroimaging of the index case (III:1) in family F13 displaying progressive calcifications in the cerebellum, thalamus, basal ganglia (lentiform and caudate nuclei) and frontal white matter. On the left axial (upper and middle panel) and sagittal sections (lower panel) from the first CT scan at age 27 and to the right corresponding scans performed 5 years later.



**Figure 5:** Neuroimaging of a patient carrying the R467X mutation in the *SLC20A2* gene. This patient is afflicted by ataxia and dementia. The axial CT scans (B and D) display progressive calcifications. The first CT scan (B) was performed at age 50 and the second (D) three years later. Calcifications in the cerebellar dentate nuclei are confluent and merge in the vermis (A and B). Symmetric and dense calcifications are also widespread in the thalamus, lentiform and caudate nuclei with milder calcifications in the occipital sulci.

Ataxia, subcortical dementia, dystonia and supranuclear palsy were the main features of this phenotype. This patient also suffered from psychiatric disorders (restlessness, lack of insight and delusions). The TCS was high, in line with previous findings in carriers of *SLC20A2* gene mutations as compared to other forms of PFBC. These calcifications were found to be progressive by coregistration, but not TCS. Her CSF-NfL was elevated 6-fold; in addition her CSF-phosphate (CSF-Pi) was also elevated in comparison to seven healthy controls (41% higher) and one male patient from the F13 family (III:2). Elevated CSF-Pi and progressive brain calcifications have been demonstrated in *knockout* and haploinsufficient *SLC20A2* +/- mice (200–202). Data on CSF analyses is very scarce in PFBC, Manyam and colleagues found elevated homocarnosine in 2 patients belonging to a Canadian family that has been described in several articles and later found to harbor mutations in both *SLC20A2* and *THAP1* (203,204). Hozumi and colleagues analyzed metals in three patients with calcifications but these patients lacked a family history of brain calcifications (205). In both cases described in **Papers V** and **VI**, CSF-NfL was elevated lending support to the notion that PFBC are neurodegenerative diseases. There is a discrepancy between the increased BBB permeability demonstrated in the hypomorphic *PDGFB<sup>ret/ret</sup>* mouse model (195,197) and the CSF findings in patients of the F13 family. Whether the permeability of the BBB changes with age and/or depend on the underlying mutation type remains to be studied. **Paper VI** suggests that elevated CSF-Pi may be a potential biomarker for PFBC associated with PFBC.

Both radiological and clinical penetrance has been described in PFBC. The radiological penetrance is very high but the clinical penetrance reduced and estimated to be around 60% (132,133) which may well be an underestimation since several features are subtle. Only detailed evaluation revealed significant cognitive deficits and anomia in two of our patients (**Paper V**). Clinico-anatomical correlations are difficult to establish clearly in PFBC. For instance, ataxia was more common among patients with calcifications in the basal ganglia, but not in the cerebellum.

The frequency of symptoms and signs reported in genetically proven PFBC cohorts and in one systemic review varies widely; from 14-61% for movement disorders, 15-59% for cognitive impairment, 15-76% for psychiatric symptoms and 20-36% for migraine/other headaches (132,133). In that systemic review, mean AO for *SLC20A2* and *PDGFRB* mutation carriers was 31.7 and 16.3, respectively. However, incomplete data is highlighted as a limiting factor in the analysis (133).

Although the significant overlap in clinical presentation makes diagnosis of PFBC challenging, certain conclusions can be drawn. First, calcifications associated with *SLC20A2* mutations are more severe and widespread than those for other PFBC forms. Such calcifications are detected in the cortex and vermis of most carriers of *SLC20A2* mutations. Calcifications are milder in *PDGFRB* mutation carriers. Migraine is common in *PDGFB*- and *PDGFRB*-associated PFBC; however this feature did not segregate well in the latter group. Patients with *XPR1* mutations may be more affected by cognitive decline (131,132,206,207).

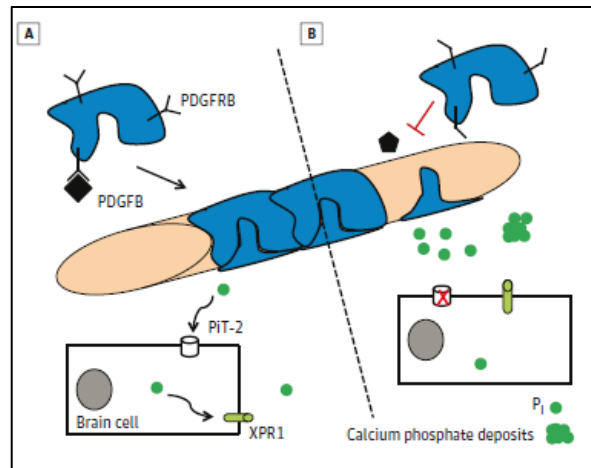
Neuropathology has provided insights into PFBC, but to date only two genetically-proven *SLC20A2* mutation carriers have been examined in this respect. Their calcifications affected the tunica media of brain arteries and the brain parenchyma, but not the veins (198,204). In addition, immunostaining for PiT-2 was reduced and a faint signal on Western blots of this protein was evident in the frontal cortex, putamen and cerebellum (198).

Mutations in *SLC20A2*, of which more than 50 have been identified to date, are the most common cause of PFBC (208), followed by mutations in *PDGFB* (10 so far), while mutations in *PDGFRB* and *XPR1* are very rare (207,209). What do the PFBC genes have in common? The products of *SLC20A1*, *XPR1* and *PDGFRB* genes are involved in phosphate transport and homeostasis (140,141,206). Impaired BBB has been demonstrated in *PDGFB* and *PDGFRB* null and hypomorphic mice (195,210–212). *SLC20A2* is expressed ubiquitously, high levels of expression are found in the vascular smooth muscle, parathyroid glands, kidney, gut, bone and placental pericytes (213–215). *SLC20A2* is also highly expressed in the brain (neurons, endothelial cells and astrocytes), particularly in the calcified areas (214). *XPR1* is also expressed in several tissues, including the brain (216). Impaired phosphate transport and subsequent hyperphosphatemia in the CSF appear to trigger brain calcifications, at least in the *SLC20A2 knockout* mouse model. It has been proposed that calcification begins in the vessels of the brain; hyperphosphatemia in the CSF may trigger this process in a similar manner to how hyperphosphatemia due to chronic kidney failure leads to calcification of peripheral vessels (202,217). *PDGFRB* is tyrosine kinase receptor, expressed widely in the CNS (neurons, pericytes, vascular smooth muscle cells and choroid plexus) (141). *PDGFB*, the main ligand of *PDGFRB*, is a growth factor expressed in neurons and epithelial cells. *PDGFRB* is crucial for embryonic development, cell proliferation, survival, differentiation, chemotaxis and migration. *PDGFRB knockout* mice die soon after birth (218). *PDGFRB* plays also important roles in the development of blood vessel by promoting proliferation, migration and recruitment of pericytes as well as in the regulation of phosphate homeostasis

by inducing expression of the phosphate transporter 1 (PiT-1) in vascular smooth muscle cells (141). Activation by *PDGFB* is required for normal proliferation and recruitment of pericytes and vascular smooth muscle cells in the brain, lung, heart, kidney, placenta and skin (219). The mechanisms of disease in *PDGFRB*-*PDGFB*-associated PFBC are still unknown; so far the described mutations associated with brain calcifications are predicted to be loss-of-function. Functional evidence of *PDGFRB* mutations were first provided in vitro, however a hypomorphic *PDGFRB*<sup>red eye/red eye</sup> mouse model displays retinal degeneration but not brain calcifications (196,212,220). Interestingly, some mutations in *PDGFRB* that are predicted to be gain-of-function are associated with a progeria disorder called Penttinen syndrome and with an overgrowth syndrome called Kosaki syndrome (221,222). Different to the loss-of-function mutations, neither Penttinen nor Kosaki syndromes are associated with brain calcifications. A summary of the genes associated with PFBC is shown in Figure 6. Dysmorphism has only been described only in one Tyrolean family with a *SLC20A2* mutation (223,224).

The risk of incidental brain calcifications increases with age, but there are presently no validated normative data in this respect for older age groups. The higher the TCS, the more likely the patient is symptomatic (131). Recently, a predictive model for PFBC, based on age and number of symmetric calcifications, and claimed to have a sensitivity of 100% and specificity of 92% (224). Integrating this algorithm with CSF biomarkers might improve diagnostic specificity.

There is still some confusion around the nomenclature of diseases with brain calcifications, in part because of the reassignment of loci during recent years. For instance, the family with proposed linkage to chromosome 14 (14q13) was shown later to harbor a mutation in the *SLC20A2* gene which is located on chromosome 8 (139,208). Likewise, another family with proposed linkage to chromosome 2q, carried in fact, a mutation in *SLC20A2* (223,224). IBGC2 and IBGC3 have merged with IBGC1. Such reassignments illustrate the pitfalls and limitations of linkage analysis and genetic testing. The underlying genotype in several PFBC families remain to be discovered, for instance Nicolas and colleagues described 47 out of 72 French patients with probable PFBC with unknown mutation (131).



**Figure 6:** Genes associated with primary familial brain calcifications (PFBC). **A.** Two of these genes, *SLC20A2* and *XPR1*, encoded proteins involved in phosphate transport. *SLC20A2* encodes the phosphate transporter 2 (PiT-2). *PDGFB* and *PDGFRB* are involved in cell proliferation and pericyte recruitment. *PDGFRB* also induces transcription of phosphate transporter 1 (PiT-1). **B.** These mutations lead to alterations in the blood-brain-barrier, in the *PDGFB*<sup>ret/ret</sup> mouse model, but not the F13 family, and impaired phosphate transport respectively. Hyperphosphatemia in the CSF occurs in *knockout SLC20A2* mice. Figure from V. Tadic et al in Primary familial brain calcification with known gene mutations: a systematic review and challenges of phenotypic characterization. JAMA Neurol. 2015 Apr;72(4):460–7. Reproduced with permission.

The studies described in **Papers IV, V and VI** have certain limitations. First, important clinical data was missing for some included in the *PDGFB* cohort. For example, AO was available for only 15 out of the 31 patients in the families included. Furthermore, information was available on only two of the four patients from family 10, one affected by headaches did not undergo a brain CT scan and the possible symptoms of another relative with brain calcifications were not reported. Lack of cognitive assessment of the men from the F13 family also limits generalizations concerning the L9R *PDGFB* mutation. The discrepancy between the oxysterol findings on the hypomorphic *PDGFB*<sup>ret/ret</sup> mice and the patients from the F13 family described above remains to be resolved. Finally, generality of CSF-Pi in *SLC20A2* mutations cannot be claimed until tested in other PFBC patients.

## 12.8 Neurological features of two Swedish Gaucher disease cohorts (paper VII)

In **Paper VII** a wide phenotypic heterogeneity in 2 Swedish GD cohorts is described (225). All but one of the patients in the GD3 cohort (n = 12) were homozygous for L444P *GBA1*



mutations, whereas the GD1 cohort (n = 13) was far more diverse. None of the GD1 patients carried biallelic L444P mutations; in the GD1 group, the N370S mutation was most common. Two patients with PD were identified in the GD1 cohort, but not in the GD3 cohort. Asymmetric rigidity, hyposmia and good L-DOPA response was identified in one GD3 patient who went through <sup>123</sup>FP-CIT SPECT. This test demonstrated reduced DAT binding in the putamen. Furthermore, mild-to-complete hyposmia, a common non-motor sign in PD, was present in 5 GD1 and 6 GD3 patients. Absence of clear Parkinsonism cases in the GD3 cohort is in contrast with a recent screening in a large PD cohort in Sweden (1625 patients), which identified a significant association with the L444P mutation and the E326K variant. The association with L444P was particularly strong in patients from the northern regions (a frequency of 4.11%) than in the country as a whole (frequency of 2.20%) (226).

Some features in the GD3 cohort are noteworthy, e.g. long survival with six of these patients living beyond the age of 40. Facial dystonia and blepharospasm were documented in four patients while the EMG of several muscle groups, including the face, was normal in two. In addition to the features typical of Norrbottnian GD3 type (truncal and appendicular ataxia, OMA, variable cognitive impairment and kyphosis), six patients exhibited focal epilepsy. However, none of the patients in these cohorts had myoclonic epilepsy as otherwise described in other GD3 cohorts (155). Anxiety was more common in the GD1 cohort while impaired cognition was evident in 4 GD1 patients and 4 GD3 patients.

All but two of the GD3 patients underwent splenectomy and all but two were treated with ERT, the remaining two receiving bone marrow transplantation (BMT). Treatment regimens in the GD1 cohort were more variable, ERT was given most commonly alone (4) or in combination with SRT (2). SRT alone was administered to one patient, another was treated with BMT and 5 patients did not receive any treatment at all in the remaining GD1 cohort. Surprisingly, no members of either of these GD groups demonstrated detectable neurological progression in the course of 3 years (cognition or movement disorders). Optimization of anti-epileptic medication may have influenced this outcome. In contrast, the European GD3 cohort showed progression over the course of 4 years (167).

The genotype-phenotype correlations associated with GD-PD are complex; at least a correlation with gene dose has been established in cases of parkinsonism associated with GBA1 mutations. The GD-PD phenotype (either homozygous or compound heterozygous mutations) is characterized by earlier AO and more severe non-motor features (psychiatric

symptoms, dysautonomia, RBD and hyposmia) as well as faster motor progression and more severe cognitive decline than in the case of carriers of heterozygous *GBA1* mutations and patients with iPD (158,164). Intriguingly, the E326K and T369M *GBA1* variants recur in association with PD but not with GD, implying that the mechanisms underlying GD and PD differ (227).

The mechanism underlying GBA-associated parkinsonism is unknown, although a bidirectional loop connecting GCase and  $\alpha$ -synuclein has been proposed (228–230). Deficient GCase was found to induce increased accumulation and toxicity of  $\alpha$ -synuclein *in vitro*, then in GD mouse models and later in the brains of PD patients with and without *GBA1* mutations (161,229,231,232). Recent evidence indicates that this toxicity impairs simultaneously time trafficking of both GCase and other lysosomal hydrolases (229)). However, the majority of GD patients and heterozygous GBA mutation carriers do not develop parkinsonism. Investigation of the potential role of gene modifiers and other interactions (gene-environment) will help clarify the variable expressivity of GD and GBA-associated parkinsonism.

Small sample size is once again a limitation here precluding definitive conclusions concerning the long-term prevalence of PD in our two GD cohorts. The Norrbottnian GD3 cohort represents the vast majority of GD3 patients in Sweden and the GD1 cohort represents approximately half of all the GD patients in the country. In addition, the follow-up time seems short as compared with a larger study on GD patients from Central Europe (167). The EMG of the cases studied ruled out myokymias and myopathies, although the phenomenology presented with concomitant blepharospasm is more compatible with facial dystonia.

### 13. CONCLUSIONS

The following conclusions can be made for this thesis:

- The Swedish family affected by inherited prion disease (IPD) with 8-OPRI (HDL1) displays an unusually long survival.
- There is an inverse correlation between early AO and long survival in IPD with 8-OPRI.
- Polymorphism in codon 129 of PRNP exerts a major impact on AO and survival in IPD with 8-OPRI, accounting together with gender, for as much as 50% of variability in AO.
- Diagnostic clues for AOA4 include the presence of obesity and biochemical abnormalities like reduced levels of albumin and elevated levels of AFP and cholesterol.
- A description of a Swedish patient with complex hyperkinetic syndrome associated with mutations in PNKP gene strengthens AOA4 as a novel entity. Novel features of AOA4 identified in the Swedish patient are the presence of chorea and low levels of PNKP protein in blood.
- Spinocerebellar ataxia type 4 (SCA4) in two Swedish families is associated with novel features including dysautonomia, dystonia, small fiber polyneuropathy and widespread neurodegeneration.
- Anticipation is also suggested for one Swedish SCA4 family for which the underlying mutation in SCA4 remains to be discovered.
- Mutations in the PDGFB gene account for the second most common form of primary familial brain calcifications.
- Signs and brain calcifications are progressive in a family with the L9R mutation in PDGFB. This family does not display the impaired blood-brain-barrier detected in the hypomorphic PDGFB ret/ret mouse model.
- Ataxia, dementia, supranuclear palsy and widespread brain calcifications are associated with the R467X mutation in SLC20A2 in a Swedish patient. Similar to the animal SLC20A2 knockout mouse models level of phosphate in her CSF was elevated
- Parkinsonism was present in a Gaucher disease type 1 (GD1) cohort but not in the Norrbottnian Gaucher disease type 3 (GD3) cohort. Some of the latter display features that may indicate presymptomatic parkinsonism (e.g., hyposmia, abnormal DAT-scan).

- During a three-year period there was no detectable progression in either if of these cohorts. The Norrbottnian GD3 cohort displays unusually long survival, as well as dystonia.

## 14. FUTURE DIRECTIONS

Mass sequencing has revolutionized medicine in the last years, allowing what were previously challenging, expensive and time-consuming investigations to be performed much more readily, as illustrated in some of the articles included here. A remaining challenge is to connect the growing number of genes shown to be involved in neurodegeneration with their convergent pathways. There is also a compelling need to identify validated biomarkers and at last improve current treatments. To our surprise, CSF-14-3-3 which is widely used as a diagnostic tool for sCJD has not been evaluated in this respect for IPD with 8-OPRI. Evaluation of *PRNP* polymorphisms other than codon 129, identification of the prion protein strain and transmission studies in IPD with 8-OPRI remain to be done. Our own research group is presently carrying out a local HDL screening.

Several questions concerning *PNKP* mutations remain to be answered. For instance, we do not know whether there is a correlation between the level of PNKP enzyme activity and/or protein levels and the severity of AOA4 and MCSZ. There is evidence for enhanced neuronal apoptosis in MCSZ at least during embryogenesis, but is this also the case for AOA4? The neuropathology for AOA4 also remains to be studied and an animal model is needed. Whether the ataxia syndromes associated with genetic defects in SSBR converge with regards to pathogenesis/disrupted hubs is unknown. It is striking that defects in SSBR do not predispose for cancer in the same manner as for diseases with defective DNA damage signaling (double-stranded break signaling) like A-T and NBS.

A thorough analysis of chromosome regions other than 16q in SCA4 patients is necessary. IHC analysis of the GABA-ergic and other neurotransmitter systems is ongoing in our laboratory and possible correlations with the abnormalities observed by PET imaging will be evaluated. SCA4 provides a unique model to study ataxia and dysautonomia. Dissection of the neuronal networks disrupted in this disease will provide knowledge for dysautonomia that may also be relevant to more common conditions like PD and MSA.

Despite major and rapid advances in genetic discoveries, many questions have arisen in connection with PFBC. We still do not know at what age brain calcifications emerge or their long-term rate of progression. Longitudinal studies will help to determine the rate of clinical progression and penetrance. The value of elevated levels of CSF-Pi as a potential biomarker requires assessment of large PFBC cohorts. In addition, the neuropathology of *PDGFB*- and *PDGFRB*-associated PFBC remains to be analyzed; only two cases harboring

*SLC20A2* mutations have examined to date. Also unknown is the role of gene modifiers and/or other potential compensatory mechanisms underlying the reduced clinical penetrance of PFBC. Characterization of the *PDGFB*<sup>ret/ret</sup> mouse model with respect to movement and/or behavioral abnormalities will be hard to interpret due since the BBB damage exhibited by this model is not detected in patients harboring the L9R *PDGFB* mutation. The algorithm for prediction of PFBC proposed by Grütz requires validation (224) which we will apply to evaluate brain calcifications in CT scans performed at our hospital in connection with characterization of a local PFBC cohort.

Finally, the complex association between *GBA1* mutations and parkinsonism will require assessment of first degree relatives to patients in the Swedish GD cohorts. The role of epigenetic factors in GBA-associated parkinsonism have not been assessed in detail yet. Last but not least, there is a need for establishing/defining biomarkers for this association.

## 15. POPULÄRVETENSKAPLIG SAMMANSTÄLLNING

I denna avhandling beskrivs några sällsynta ärftliga neurologiska sjukdomar som huvudsakligen påverkar motoriken. I studie I beskrivs en ärftlig prionsjukdom som går med ofrivilliga rörelser, personlighetsförändring, varierande förekomst av psykiatriska symtom och även demensutveckling. Ofrivilliga rörelser i denna sammanställning inkluderar chorea (snabba och slumpmässiga rörelser) och dystoni (ihållande sammandragning av olika muskelgrupper). Ett annat motoriskt fenomen som tas upp i avhandlingen är ataxi. Ataxi är en medicinsk term som beskriver nedsatt koordination och obalans vilket oftast uppstår till följd av skador i lillhjärnan. Vanligaste orsaken till ärftlig chorea är Huntingtons sjukdom också kallad danssjuka. Liknande symtombild förekommer vid andra ärftliga tillstånd som kallats för "Huntingtonliknande sjukdomar" (Huntington's disease-like, HDL på engelska). Det finns flera olika typer av ärftliga prionsjukdomar, en av dem kallas för Huntingtonliknande sjukdom 1 (HDL1). Det har beskrivits fyra olika familjer med denna sällsynta sjukdom, en av dem var från Sverige. Alla individer i den svenska familjen är avlidna. Symtombilden vid HDL1 består av beteendestörningar, ofrivilliga rörelser, ataxi och tidig demensutveckling. Data från alla fyra familjer med denna sjukdom samlades och analyserades. Dels visas i denna studie att en annan förändring i prionanlaget påverkar HDL1. Den svenska familjen hade den längsta överlevanden av alla fyra familjerna. Tidigare ålder vid insjuknandet korrelerade med långt förlopp vid HDL1.

Studie II i avhandlingen berör en komplex och relativt nybeskriven sjukdom vid namn ataxi med oculomotorisk apraxi typ 4 (AOA4). Oculomotorisk apraxi betecknar svårigheter att föra blicken åt sidorna på uppmaning. Ett fenomen som sticker ut med AOA4 är övervikt hos en tredje del av patienter och avvikande laboratorieprover i form av låg proteinnivå men stegrade kolesterol- och AFP-värden. AFP står för alfafetoprotein. En svensk patient med fortskridande symtombild i form av ataxi, uttalad polyneuropati (påverkan på perifera nerver) och kognitiv påverkan beskrivs. Symtomdebut skedde i barndomen, MR-hjärna påvisat i detta fall fortskridande atrofi (skrumpning i lillhjärnan). Nya drag för AOA4 i det beskrivna fallet är förekomst av chorea under barndomen, långsam sjukdomsutveckling och låga halter i blodet av proteinet som är muterat. Beskrivningen stödjer uppfattningen att AOA4 är en ny och distinkt sjukdom.

Spinocerebellär ataxi typ 4 (SCA4) är en sällsynt sjukdom som beskrevs för första gången hos en skandinavisk familj i den amerikanska mellanvästern 1996. Genetiska studier visade att

SCA4 var kopplad till beskrevs en region på associerad till kromosom 16q22.1. En annan SCA4 familj med koppling till samma region på kromosom 16 rapporterades några år senare i Tyskland. Symtombilden vid SCA4 består av debut i vuxen ålder, ataxi och polyneuropati. SCA4 är en långsamt fortskridande sjukdom. I studie III i denna avhandling beskrivs två svenska SCA4 familjer med bredare och mycket varierande symtombild än vid föregående beskrivningar. Hos majoriteten av undersökta patienter påvisades en varierande grad av dysautonomi först. Dysautonomi innebär störning i funktioner som inte styrs viljemässigt såsom bl.a. reglering av blodtrycket vid lägesändringar, andningsfunktionen i sömnen, och tarmfunktionen. Dysautonomi förekommer vid andra SCA former men är inte lika utbredd och vanlig som vid SCA4. Andra motorikstörningar än ataxi konstaterades hos några patienter i de svenska familjerna såsom dystoni. Varierande grad av atrofi (skrumpning) i lillhjärnan, hjärnstammen och ryggmärgen påvisades i alla de testade patienterna. Andra bildavgivande metoder visat generell nedsatt ämnesomsättning i lillhjärnan. En utbredd neurodegeneration d.v.s. förlust av flera olika typer av nervceller påvisades i två fall, detta drabbade lillhjärnan och oväntad nog även ryggmärgen. Kandidatregionen i kromosom 16 blev avläst men ingen uppenbar sjukdomsorsakande förändring kunde påvisas.

Förekomst av förkalkningar i hjärnan kallas vanligen för ”Fahr’s disease”. Det finns en rad olika orsaker till förkalkningar i hjärnan. En ovanlig form av detta tillstånd utgörs av en grupp ärftliga sjukdomar som samlas under namnet primära och familjära hjärnförkalkningar (PFBC engelsk förkortning). Symtombilden vid PFBC är mycket varierande. Studie IV handlar om upptäckten av mutationer i en tillväxtfaktor för kärl som orsak till förkalkningar i hjärnan hos sex obesläktade familjer från olika länder. En av dessa familjer var en svensk familj som utretts på neurologmottagningen. Familjen består av fyra patienter med förkalkningar i hjärnan, migrän och ofrivilliga rörelser i form av skakningar och chorea. Hos denna och de fem andra familjerna upptäcktes förändringar i tillväxtfaktorn som orsak till förkalkningarna. En genmanipulerad musmodell för denna sjukdom analyserades. Denna musmodell uppvisar fortskridande förkalkningar i hjärnan och en ökad genomsläpplighet i sin blod-hjärnbarriär. I studie V gjordes en uppföljning av familjen med förkalkningar i hjärnan. Hos samtliga patienter konstaterades antingen tillkomst av mild obalans eller fortskridande ofrivilliga rörelser. Förkalkningarna i hjärnan hade också ökat hos 3 av 4 patienter. I studien analyserades ryggvätskan från tre patienter i den svenska familjen och jämfördes med musmodellen för denna sjukdom. Till skillnad från musmodellen var blod-hjärnbarriär hos patienterna normal. I studie VI beskrivs en patient drabbad av ataxi, personlighetsförändring,



nedsatt minne och omfattande förkalkningar i hjärnan. Förkalkningarna var fortskridande även i detta fall. Utredningen påvisade en mutation i en gen viktig för fosfattransport i kroppen. En genmanipulerad mus används som modell för denna sjukdom och som uppvisar stegrad fosfatnivå i ryggvätskan. Liknande avvikelser konstaterades hos patienten. I båda studierna (V och VI) tillämpades en analysmetod för att utvärdera om förkalkningarna i hjärnan ökat.

Gaucher sjukdom (GS) är en autosomal recessiv sjukdom som uppstår till följd av mutationer i *GBA* genen och påverkar framför allt blodbildningen. GS delas upp i tre olika typer utifrån förekomst av neurologiska symtom: GS typ 1 (GS1) är absolut vanligast och traditionellt sett inte associerad med neurologiska symtom. GS typ 2 (GS2) är en mycket svår form som drabbar barn och GS typ 3 (GS3) som är associerad med neurologiska symtom. En särskild form av GS3 finns i Norrbotten där majoriteten av alla patienter bär på samma sjukdomsanlag i dubbeluppsättning. Mutationer och varianter i enkel eller dubbel uppsättning i *GBA* genen utgör också en betydande riskfaktor för Parkinsons sjukdom och liknande tillstånd. I studie VII undersöktes en grupp patienter med GS1 (n = 13) och en grupp patienter med GS3 (n = 12). I GS1-gruppen identifierades fall av Parkinsons sjukdom men inga alls i GS3-gruppen. Några patienter hade nedsatt lutförmåga vilket kan föregå Parkinsons sjukdom. Sex GS3 patienter har levt över 40 år vilket är ovanligt. Dystoni dokumenterades som ett nytt motorikfenomen i GS3-kohorten. Ingen grupp hade detekterbar neurologisk progression under loppet av 3 år.

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